1
Amino Acids

BY G. C. BARRETT

1 Introduction

All major sub-divisions of amino acid science are represented in this Chapter as in previous Volumes of this Specialist Periodical Report (formerly named 'Amino acids, Peptides, and Proteins'), though with some waxing and waning as topic areas develop or become exhausted. The emphasis continues to reside in chemical studies but covers the biological literature to the extent that chemical and analytical studies are included there.

2 Textbooks and Reviews

Reference texts[1] and compendia of data[2,3] include the second supplementary list of amino acid derivatives that are useful in peptide synthesis (taking in the literature to the end of 1982).[3] Other topics reviewed include 1-aminocyclopropanecarboxylic acid,[4] synthesis of N-methylamino acids,[5] applications of uncommon amino acids in natural-products synthesis,[6] the role of S-adenosylhomocysteine[7] and of L-ergothioneine,[8] and boron analogues of amino acids[9] including p-borono-L-phenylalanine.[10]

3 Naturally Occurring Amino Acids

3.1 Occurrence of Known Amino Acids.- Close relatives of the common amino acids are covered here, and no attempt is made to review the routine literature of the distribution of well-known amino acids.

The first natural appearance of methionine sulphoximine is reported;[11] it is the toxic principle of Chestis glabra. L-DOPA-3-Sulphate has been located in the brown alga Ascophyllum nodosum,[12] and α-hydroxymethylserine, not previously reported to be a natural product, has been found in Vicia pseudo-orobus.[13]

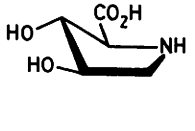
N-Substituted amino acids continue to arise in a variety of systems: N-trimethylalanine at the N-terminus of myosin light chains,[14] L-pyrrolidone-2,4-dicarboxylic acid in the muscle of the mollusc abalone Haliotis discus hannai,[15] and 3R,4R-dihydroxy-L-proline (1), present in virotoxins.[16] (1), like (+)-3,4,5-trihydroxypipecolic acid (2) from Baphia seeds,[17] competitively inhibits cattle β-D-glucuronidase.[16] Leucinopine, one of a group of N-(1-carboxyalkyl)amino acids often categorized as 'opines', has been shown[18] to possess the L-threo stereochemistry; in other words, this amino acid, N-(1,3-dicarboxy-

propyl)leucine, has the 'L-glu-,L-leu-' configuration and in this respect is unique amongst the other opines octopine ('D-ala-,L-arg-'), nopaline ('D-glu-,L-arg-'), and succinamopine ('D-glu-, L-asn-').[18]

Plant, fungal, and microbial sources of less common amino acids: Asplenium unilaterale (4-hydroxy-L-2-aminopimelic acid as well as D-2-aminopimelic acid and trans-3,4-dehydro-D-2-aminopimelic acid),[19] Dactylosporangium aurantiacum (L-threo-β-hydroxyaspartic acid, previously only found in Arthrinium and in various Streptomycetes),[20] and Amanita pseudoporphyria (L-2-aminopent-4-ynoic acid and L-2-aminopent-4-enoic acid, L-2-aminohex--4-ynoic acid and L-2-aminohept-4-en-6-ynoic acid, as well as L-2-amino-4-chloropent-4--4-enoic acid and L-2-aminohexa-4,5-dienoic acid as previously reported).[21] Another cyclic tetrapeptide from Helminthosporium carbonum has been described,[22] containing a 2-amino-8-oxo-9,10-epoxydecanoic acid residue. L-Phenylalanine and its 3S-methyl homologue (3) occur as their N-acetyl derivatives esterified with the unusual 8R-hydroxy-9S-methyl oxiranyl-2E,4Z,6E-decatrienoic acid as AK-toxins I and II from Alternaria alternata pear fungus (black spot disease).[23]

Cross-linking amino acid residues in mammalian proteins continue to attract interest, a recent citation referring to the identification of pyridinoline in Type 1 collagen.[24]

3.2 New Natural Amino acids.- New aliphatic α-amino acids include 2S-aminohex-5-ynoic acid (from Cortinarius claricolor var. tennipes),[25] D$_s$-erythro-2-amino-4-ethoxybutanoic acid (from the edible mushroom Lyophyllum ulmarium),[26] erythro-γ-hydroxyhomo-L-arginine (from the seed of Lonchocarpus costaricaensis; the threo diastereoisomer is already known to be a natural product),[27] and the sulphate ester of trans-4-hydroxypipecolic acid (seeds of Peltophorum africanum).[28] This is the first naturally occurring sulphate ester of a non-protein amino acid to be reported. The bulgecins contain O-glycosylated 5-hydroxymethyl-4-hydroxyproline amides (4; R = glycosyl residue).[29,86] 'Dealanylalahopcin' (5), found with alahopcin in Streptomyces albulus cultures, is (2S,3R)-2-amino-4-formyl-3-(hydroxyaminocarbonyl)butyric acid[30] (wrongly named as the 4-(hydroxyaminocarbonyl)acid in the original paper). A high level of interest in N-(1-carboxyalkyl)amino acids (the 'opines'; see listing in preceding Section) is reflected in three new examples from the 1985 literature: crown-gall tumours incited by Agrobacterium tumefaciens produce agropine and related mannityl opines and leucinopine and in addition large amounts of a new member of the family N-{(1S)-1-carboxy-2-carbamoylethyl}-(S)-glutamic acid ('LL-succinamopine').[31a] The D,L-diastereoisomer having been isolated previously from the same source, this is the first example of the natural occurrence of epimeric opines. The other two new opines are N-(1-carboxyethyl)-L-methionine[31b] and a phosphorylated example (agrocinopine A) secreted by healthy crown-gall cells induced by the same bacterium.[32]



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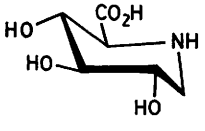

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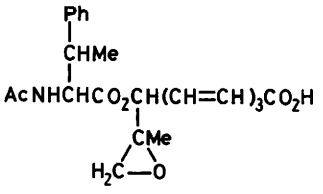

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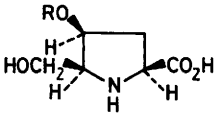

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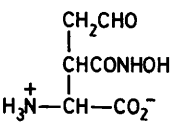

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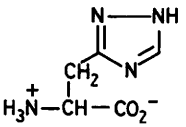

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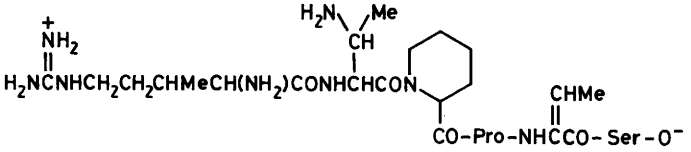

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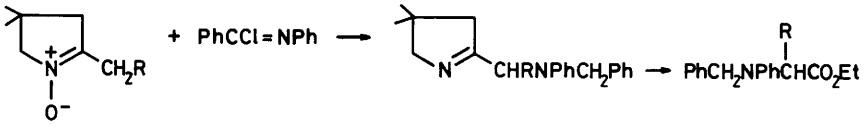

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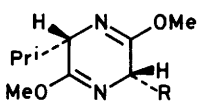
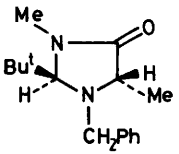


(9) R = H
(10) R = Me



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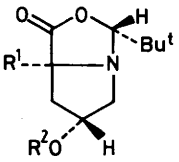

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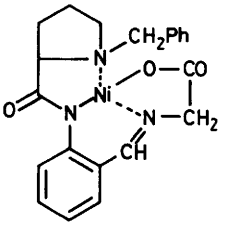

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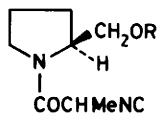

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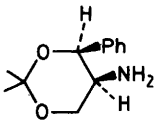

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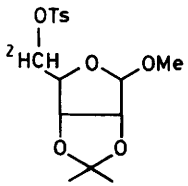

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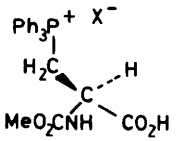

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Streptomyces KM-10329 produces β-(1,2,4-triazol-3-yl)-L-alanine (6).[33]

3.3 New Amino acids from Hydrolysates.- This section specifically refers to natural products that in principle or in practice can release new amino acids on hydrolysis.

Lavendomycin from Streptomyces lavendulae is an unusual peptide (7) containing some close analogues of common amino acids.[34]

Carzinophilin contains (2S,3S)-4-amino-2,3-dihydroxy-3-methylbutanoic acid.[35]

4 Chemical Synthesis and Resolution of Amino Acids

4.1 General Methods of Synthesis.- The major standard methods, mostly of many years' standing, continue to be fully used. It is not necessary to do more than cite most of these with literature references (recent review coverage is available[36]), and some further details of the synthetic objectives are given in later sections of this Chapter. The alkylation methods in which the side chain of the α-amino acid is put in place include alkylation of acetylaminomalonates[37] and other glycine derivatives $(MeS)_2C{=}NCH_2CO_2Et$,[38] $Ph_2C{=}NCH_2CO_2Et$,[39,40] $PhCH{=}NCH_2CO_2Et$,[41] $CNCH_2CO_2Et$,[42] and azlactones.[43]

Alkylation of methyl 2-acetamidoacrylate with a Grignard reagent in the presence of copper(I) iodide gives moderate yields of 3-substituted alanines,[44] and N-alkylamino acid esters, benzaldehyde, and alkenes react in refluxing toluene to give prolines.[45] The latter process involves cycloaddition to intermediate azomethine ylides.

Strecker synthesis of α-amino nitriles[46,47] involving reaction of an aldehyde, a secondary amine, and Me_3SiCN in MeOH can be accomplished within less than 5 minutes, thus providing some assistance in the synthesis of α-amino acids labelled with short-lived radioactive isotopes.[47] Dehydrogenation of aliphatic secondary amines by phenylseleninic acid (or its anhydride), under mild conditions in the presence of NaCN or Me_3SiCN, is a new route to α-amino nitriles.[48]

A full paper has been published on the synthesis of N-acyl α-amino acids through the isomerization - amidocarbonylation of allylic alcohols by primary amides and H_2 with carbon monoxide, using a homogeneous binary catalyst system $HRh(CO)(PPh_3)_3$ with $Co(CO)_8$ or $Fe_2(CO)_9$: $R^1R^2C{=}CR^3CH_2OH + RCONH_2 \longrightarrow R^1R^2CHR^3CH(NHCOR)CO_2H$.[49]

A new amino acid synthesis adding to the group of methods in which the amino function is introduced into an alkanoic acid (or a precursor of it) has been reported.[50] Ethanolysis of the pyrroline formed after reaction of the corresponding N-oxide (8) with N-phenylbenzimidoyl chloride yields an N-phenyl-N-benzylamino acid ethyl ester, from which the various N and C substituents can be removed by standard methods.

4.2 Asymmetric Synthesis.- Further examples have accumulated in the literature during 1985 to extend established methods in the amino acid area. The already voluminous output of Schöllkopf and co-workers, based on the alkylation of bis-lactim ethers (9) derived from piperazine-2,5-diones, has been augmented to include syntheses of D-tryptophan methyl ester and (R)-α-methyltryptophan methyl ester,[51] and other alkylation processes in which very high diastereoselectivity is achieved.[52-56] One of these[54] deals with asymmetric synthesis of D-threonine through reaction of acetaldehyde with the $Ti(NMe_2)_3$ complex of the bis-lactim ether. Another is concerned with the synthesis of chiral deuteriated α-aminoisobutyric acid through reaction of the bis-lactim ether (10) with C^2H_3I.[56]

Further results from Seebach's group[57-59] on the alkylation of chiral enolates with what has been called 'self-reproduction of the centre of chirality' – i.e. the incoming group takes the place of the proton that is substituted — confirm the high (>90%) diastereoselectivity that accompanies this approach. Enantiomerically pure pivalaldehyde aminals (11) derived from N-benzyl-L-alanine can be alkylated and elaborated into (R)- or (S)-α-methylDOPA, depending on the cis or trans orientation, respectively, of the aminal.[57] Other α-methyl analogues prepared in this study in high optical purity include α-methyl-L-methionine and α-methyl-L-valine. Pivalaldehyde N,O-acetals (12) from O-acyl-4-hydroxy-L-proline[58] and the corresponding compound from L-thiazolidine-4-carboxylic acid[59] have also been studied in what is clearly the start of a programme seeking to understand the relationship of structure to carbanion stereochemical integrity, in which the alkylating agent no doubt plays a role.[279]

Highest optical purity was observed in the stereoselective synthesis of L-aspartic acid through alkylation of a di-alkyl malonate with N-benzyloxycarbonyl-L-alanyl-2-chloroglycine methyl ester, followed by hydrolysis, when the malonate carried bulky alkyl groups.[60]

Other studies based on recent pioneering work include alkylation of chiral nickel(II) complexes, to yield either D-serine in better than 80% enantiomeric excess, using 0.2M NaOMe as base, but L-serine in 80-98% enantiomeric excess, using NEt_3,[61] when the complex (13) formed between N-benzyl-L-proline N-arylamide and the contiguous glycine Schiff base is alkylated with formaldehyde. A less puzzling result is seen for the corresponding alanine complex, used[62] for the preparation of α-methyl amino acids in optically pure form after separation of diastereoisomers over silica gel.

Alternative asymmetric synthesis of α-methyl amino acids has been established[63] but in much lower enantiomeric excess (31.7% for the R-enantiomer) when the alanine-based isonitrile (14) participates in Michael addition to acrylonitrile. Variable results (10-45% enantiomeric excess of the R-enantiomer)were obtained in the corresponding reaction with methyl acrylate, in relation to the asymmetric synthesis of α-methyl-D-glutamic acid and α-methyl-D-ornithine.[63]

The chiral-template approach employed in these examples underpins other examples, based on initial explorations already familiar to readers of earlier volumes of this Specialist Periodical Report. Weinges and co-workers have provided further examples of the use of the chiral 5-amino-1,3-dioxan (15) as a component for asymmetric Strecker synthesis of α-amino nitriles. (2S,4S)-(-)- and (2S,4R)-(+)-5,5,5-trifluoroleucine have been prepared in this way,[64] the latter from (R)-$CF_3CHMeCH_2CHO$ prepared from (E)-$CF_3CMe{=}CHCO_2Et$ through successive Pd-catalyzed hydrogenation, $LiAlH_4$ reduction, and resolution. D- and L-2-(2-thienyl)- and -2-(3-thienyl)glycines were prepared in an analogous fashion;[65] in both cases the α-amino nitriles were converted into the α-amino acids and the chiral dioxan moiety removed by HIO_4 cleavage. 'Chiral glycine', $H_3\overset{+}{N}{-}CH^2H{-}CO_2^-$, has been prepared from the(R)-toluene-p-sulphonyl ribofuranoside (16) through aminolysis with N_3^- followed by reduction; aminolysis with potassium phthalimide gave the N-phthaloyl derivative in better than 93% enantiomeric purity, after oxidative cleavage ($KMnO_4$).[66]

Fewer research groups are studying asymmetric hydrogenation methodology for the introduction of a chiral centre into an αβ-unsaturated α-amino acid. Stille has described the use of a catalyst system with a chiral phosphine attached to polystyrene in combination with a Rh(I) salt.[67]

4.3 Prebiotic Synthesis Models.- Interest in this topic covers broader areas than synthesis alone, and later sections cover models for chiral discrimination.

Synthesis of α-amino acids from simple compounds and elements, with energy input of the sort that might be available in Nature, has been a long-running topic. ^{60}Co-γ-Irradiation of oxygen-free aqueous HCN and NH_4CN gives mixtures of amino acids,[68] and some of these arise by further reactions involving glycine, which is a major initial product.[69] Aqueous KCN irradiated with u.v. light yields amino acids (but mainly oligoglycines), through a $HO^{\cdot}$-initiated chain reaction.[70] U.v. irradiation of solutions of bisglycinatonickel(II) dihydrate andan aldehyde yields mixtures of amino acids.[71] Formaldehyde gives mainly glycine, alanine, aspartic acid, and serine, acetaldehyde gives glycine, aspartic acid, threonine, and allothreonine, while benzaldehyde somewhat surprisingly also gives glycine, serine, and aspartic acid (with two unidentified products).[71] Glyceraldehyde reacts with ammonia in phosphate buffer at pH 7 to give alanine.[72] Seventeen amino acids, with glycine, alanine, serine, aspartic acid,and glutamic acid predominating, are formed in a radiofrequency plasma of H_2 and N_2 with cellulose as carbon source placed between the electrodes of the reaction cell.[73]

4.4 Synthesis of Protein Amino Acids and Other Naturally Occurring α-Amino Acids.- The literature supporting this Section can only be described as expansive and expanding, judging

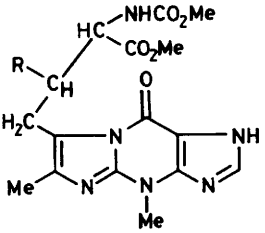
by the literature of the 1980's and particularly 1985. Frequently, the authors' primary interest is in an application of a novel synthetic procedure rather than in the establishment of a route to an amino acid for which efficient methods of synthesis already exist.

Reviews of large-scale production of protein amino acids[74,75] and peptides[75] have appeared. This topic and its fine details as represented in the biosynthesis literature can only be hinted at here, with representative citations (production of L-tryptophan and 5-hydroxy-L-tryptophan by Escherischia coli,[76] pilot-scale production of L-phenylalanine from D-glucose,[77] and L-aspartic acid production by Brevibacterium flavum[78]), with special reference to the conversion of one amino acid into another in this way (L-tyrosine and its N-formyl derivative into L-DOPA and its N-formyl derivative by Mucuna pruriens,[79] hydroxylation of phenylalanine by hypoxanthine and xanthine oxidase via H_2O_2 and the superoxide anion to give o-, m-, and p-tyrosines,[80] and DL-methionine into D-α-aminobutyric acid through the action of methionine γ-lyase and D-amino acid aminotransferase[81]).

Most of the syntheses to be described in this Section are side-chain hydroxylated amino acids; some are similarly close analogues of familiar amino acids. Few of these studies have contributed to generally applicable methodology, except the introduction of the phosphonium salt (17) as a generally useful chiral building block for β-substituted alanines. It is easily prepared from L-serine methyl ester and has been used[82] in a synthesis of S-(-)-wybutine (18), a fluorescent minor base from yeast phenylalanine tRNA through Wittig condensation with the corresponding formylpurine.

Relatively simple operations are involved in the non-enzymatic and glutamate dehydrogenase-catalyzed reduction of Δ^1-pyrroline-2-carboxylic acid, as part of a mechanistic study involving NAD(P)H and ^2H isotope effects,[83] and diborane reduction of N-benzyloxycarbonyl-α-methyl-L-glutamate to give N-benzyloxycarbonyl-L-proline methyl ester (40% yield after a 6 hour reaction in THF).[84] A much more extensive procedure is involved in the synthesis of bulgecinine (4),[29] a constituent of the bulgecins (from Pseudomonas acidophila),[85] that employs D-glucose as chiral synthon.[86] The other hydroxylated amino acids that have received attention are: (2S,3R,4R)-3,4-dihydroxyproline (stereoselective synthesis from the erythro-β-hydroxy-α-amino acid, 19) and its 2S,3S,4S-diastereoisomer[87] (the 2S,3R,4R-diastereoisomer has been prepared[88] through the separation of the mixture of stereoisomers formed through a long-established route[89]); erythro-β-hydroxy-L-aspartic acid and erythro-β-hydroxymethyl-L-serine through the common intermediate (20), formed from L-tartaric acid by oxirane ring opening and selective reduction of the resulting azido-ester;[90] and 2-amino-4-hydroxy-4-(p-hydroxyphenyl)-3-methylbutanoic acid as a mixture of stereoisomers formed through 1,3-dipolar cycloaddition of ethoxycarbonylmethane nitrile oxide to (E)-(4-methoxyphenyl)propene.[91]

(2S,4R)-Erythro- and (2S,4S)-threo-4-methylglutamic acids have been prepared[92] from



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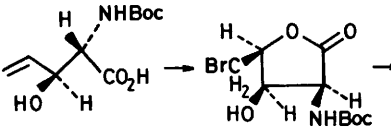

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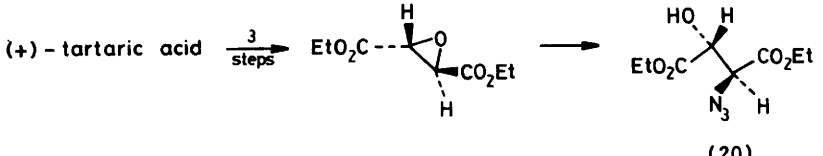

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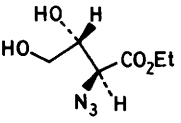

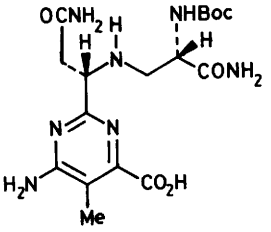

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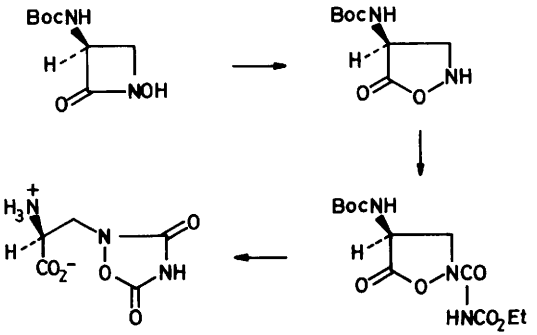
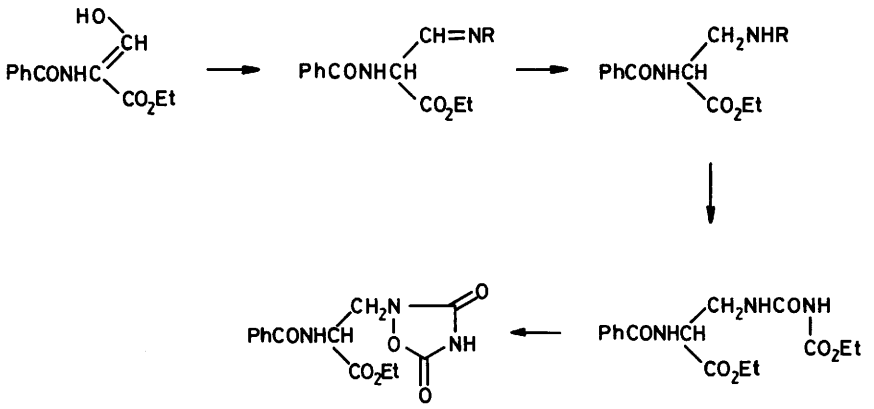
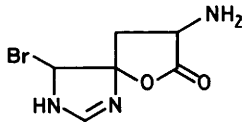


Scheme 1



Scheme 2



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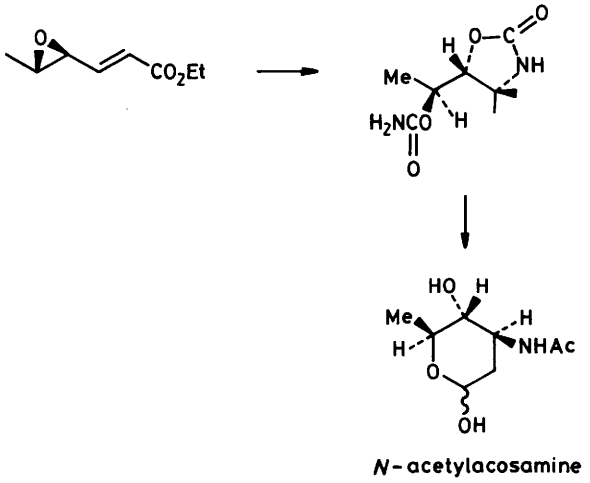


N-acetylacosamine

Scheme 3

(RS)-4-methyl-2-oxoglutaric acid by glutamate dehydrogenase-catalyzed reductive amination.

α-Amino acids with side-chain nitrogen functions that have been synthesised include N^{α}-Boc-L-2,3-diaminopropionic acid amide for use in a total synthesis of bleomycin.[93] The application of the Hofmann rearrangement to Boc-L-asparagine constituted the essential step in this synthesis. The side-chain Schiff base of this product was used in the synthesis of the bleomycin constituent pyrimidoblamic acid (21).[94] Quisqualic acid has been synthesised by routes that permit approaches to analogues at some future time: a mild new general synthesis of the isoxazolin-5-on-2-yl ring system is a notable feature of Baldwin's work (Scheme 1).[95,96] It involves a favoured 5-endo-dig cyclization and the overall route constitutes the first enantio-efficient chemical synthesis of the natural product, L-β-(isoxazolin-5-on-2-yl)-alanine, and is adaptable for the synthesis of other β-amino-alanines.[96] Bycroft's group uses a dehydroserine, PhCONHC(=CHOH)CO_2Et, as a framework on which the isoxazoline side chain is built (Scheme 2).[97]

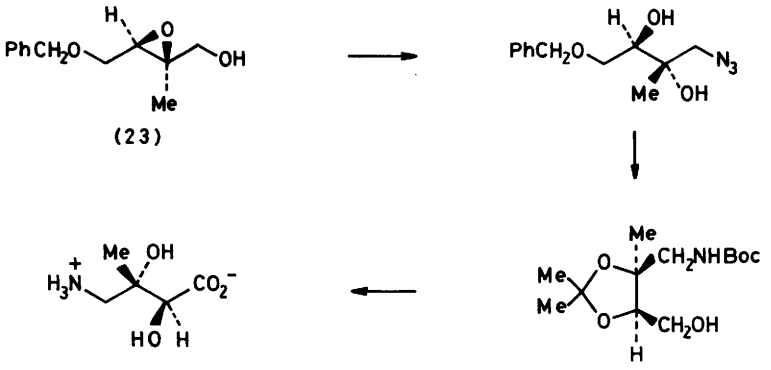
Cysteine derivatives that have been synthesized include the four stereoisomers of β-methyllanthionine, through a conventional route in which (2S,3S)- or (2R,3R)-β-methyl-cysteine is reacted with D- or L-β-chloro-alanine.[98] 2'-(S-Cysteinyl)-L-histidine, an unusual amino acid found in a tyrosinase from Neurospora crassa, has been synthesized via (22), formed from L-histidine methyl ester through reaction with Br_2.[99]

4.5 Synthesis of β- and Higher Homologous Amino Acids.- Novel oxirane ring-opening reactions are in vogue for syntheses of unusual amino acids that carry side-chain hydroxy groups (see preceding section). This is a key feature of a synthesis of N-acetyl derivatives of acosamine and ristosamine (Scheme 3).[100] Azide-ion ring opening of the oxirane (23) is also a crucial step in an efficient enantioselective synthesis of (2S,3S)-4-amino-2,3-dihydroxy-3-methylbutanoic acid, a constituent of carzinophilin (Scheme 4).[35]

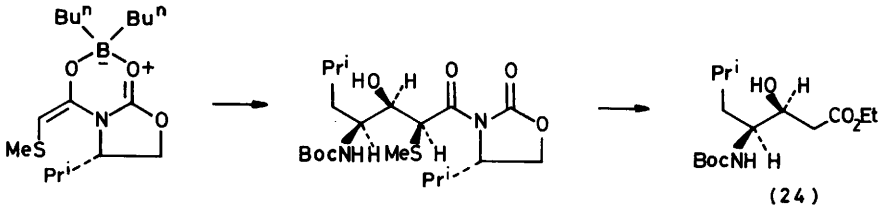
Previous syntheses of statine (24) were not diastereoselective, unlike the route shown in Scheme 5 that starts with Boc-L-leucinal and involves enantio- and erythro-selective aldol condensation with (S)-4-(1-methylethyl)-3-[(methylthio)acetyl]-2-oxazolidinone.[101] More conventional chemistry is all that is called for in syntheses of (S)-4-amino-3-hydroxybutanoic acid from (S)-malic acid via its cyclic anhydride[102] and its N-trimethyl analogue DL-carnitine, from $Me_2NCH_2COCH_3$ and diethyl carbonate in the presence of NaH, followed by reduction and N-methylation of the resulting γ-dimethylamino-β-keto-ester.[103]

Cystathionine, methionine, and lysine are utilized by Streptomyces cattleya in the formation of thienamycin (25).[104]

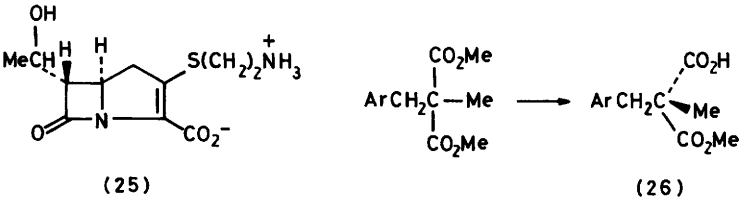
4.6 Synthesis of α-Alkyl Analogues of Protein Amino Acids.- A number of examples answering to this title have been mentioned in earlier Sections. The synthesis of α-methyl



Scheme 4



Scheme 5



amino acids through Strecker synthesis of alkylation of 1-substituted 4-methyl-2-imidazolin-5-ones has been reviewed.[105] Optically pure (S)-α-methyl analogues of phenylalanine, tyrosine, and DOPA are accessible through pig liver esterase and α-chymotrypsin-catalyzed hydrolysis of corresponding dimethyl 2-benzyl-2-methylmalonates, giving the (R)-ester (26) that is open to conversion into the acyl azide for use in a Curtius rearrangement reaction.[106]

α-Phenyl serine has been prepared by Strecker synthesis starting with $PhCOCH_2OAc$, and a similar procedure has given α-hydroxy-α-phenyl-β-alanine.[107]

4.7 Synthesis of Other Aliphatic and Alicyclic Analogues of Protein Amino Acids.- To the extent that amino acids under this heading may in due course be found in Nature, and that synthetic methods described here are equally applicable to examples found elsewhere in this Chapter, the reader seeking to know the currently used range of synthetic methods will need to read the whole of Section 4. The overlap is apparent in a use of the N-benzyloxycarbonyl Schiff base $ZN{=}CHCO_2Me$ (see also Section 4.1) for cycloaddition to cyclopentadiene to give 2-azabicyclo[2.2.2]heptane-3-carboxylic acid,[108] and a similar new proline synthesis using the azomethine ylide formed from $Me_3SiCH_2N{=}CHCO_2Me$.[109] Another route to alkylprolines[110] is based on copper(II)-catalyzed Michael addition of nitroacetic esters to αβ-unsaturated ketones, followed by reductive cyclization (H_2/Pd-C) of the resulting 2-nitro 5-oxo-esters.

Specific functional-group chemistry is involved in an improved preparation of L-homoglutamic acid from N-acetyl-L-lysine ethyl ester, using ^tBuOCl for N-chlorination, followed by dehydrochlorination and hydrolysis.[111] More general methodology is used for the conversion of αε-di-aminopimelic acid into α-amino-ε-ketopimelic acid by transamination with pyridoxal.[112]

4.8 Synthesis of α-Alkoxy-α-Amino Acids and Related α-Hetero-atom Substituted α-Amino Acids.- α-Substitution of Schiff bases $Ph_2C{=}NCH_2CO_2Et$ through 'allylic' bromination with N-bromosuccinimide in the presence of simple heteroatom nucleophiles in DMF gives the title compounds (e.g. NaOAc yields the α-acetoxy Schiff base).[40] These are valuable as electrophilic glycine synthons, $RC(NHR)CO_2R$; they react readily with organocopper reagents to give the corresponding 1-naphthyl, 2-thienyl, and t-butyl amino acids, for example.[40] A similar outcome but involving the nucleophilic glycine synthon $(MeS)_2C{=}N{-}\bar{C}H{-}CO_2Et$,[38] has been reported, reaction with aromatic aldehydes giving oxazolines.

4.9 Synthesis of Halogeno-alkyl Amino Acids.- Interest is particularly high in fluorine analogues of the protein amino acids, associated with their potential as enzyme inhibitors. β-Fluoro-α-amino acids have been reviewed.[113]

Synthesis of trifluoroalanine[114] proceeds through an unusual oxazole synthesis (Scheme 6). Other syntheses either employ variants of standard amino acid syntheses [erythro- and threo-β-fluorophenylalanines from a glycine Schiff-base benzyl ester with α-bromo-α-fluorotoluene;[115] amination of (E)-CHF=CMeCHBrCO$_2$Et, made from Me$_2$C=CHCO$_2$Et, to (E)-β-fluoromethyleneglutamic acid[116] in a route which can be used[117] to give (E)-$H_3\overset{+}{N}CH_2C(=CHF)CO_2^-$, capable of inhibition of GABA transaminase[117]] or involve manipulation of side-chain functional groups (erythro- and threo-β-fluoroglutamic acids from N-acetyl-β-hydroxyglutamic acid through fluorodehydroxylation[118]).

4.10 Synthesis of Aliphatic Amino Acids Carrying Side-chain Hydroxy Groups.- Frequent mention has been made in the preceding Sections of amino acids of this type, whether as new natural products or as compounds useful in synthesis.

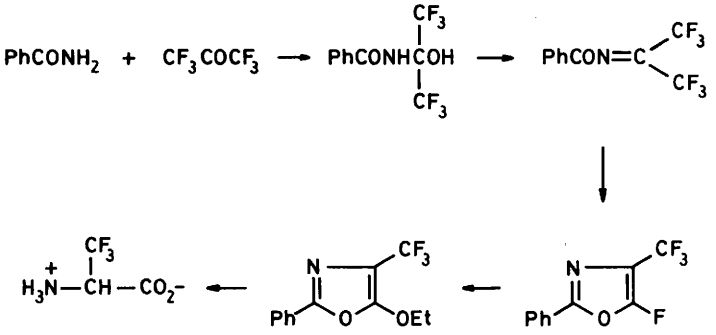
α-Aminosilylketene acetals provide β-hydroxy amino acids through Lewis acid-catalyzed addition to aldehydes (Scheme 7).[119] The 'bislactim ether' route (see Section 4.2) has been used for the preparation of β-hydroxy-γ-azido-L-valine (reaction of (27) with N_3^-).[120]

4.11 Synthesis of Aliphatic Amino Acids with Unsaturated Side chains.- While interest continues in routes to 'dehydro amino acids' (i.e. αβ-unsaturated α-amino acids), there is also increasing attention being given to homologues where the unsaturation is either further away from, or placed between, the amino and carboxy groups.

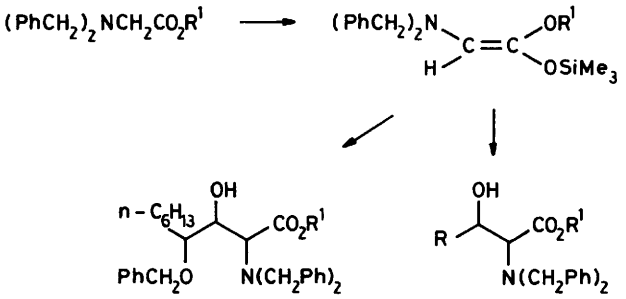
Protected dehydroglutamic acids have been prepared from corresponding α-ketoglutaric acids by condensation with benzyl carbamate.[121] An alternative approach[122] is based on the addition of malonic esters to nitriles catalyzed by tin(II) chloride, (28)→(29).

Protected βγ-unsaturated α-amino acid esters can be formed by oxidative rearrangement of γ-phenylseleno-αβ-unsaturated esters in the presence of an alkyl carbamate (Scheme 8). The route[123] has been used for the preparation of (±)-vinylglycine in 48% yield after removal of protecting groups by acid hydrolysis. An intermolecular ene reaction (Scheme 9) between allylglycine and ethyl glyoxylate shifts the unsaturation to the βγ-position and has been used[124] to prepare representative 2,6-disubstituted aminopimelic acids. A similar outcome is achieved[125] in the rearrangement of β-hydroxyallylglycine (30), obtained through SeO_2-^{t}BuOOH oxidation of protected allylglycine.[126] The acetate of (30) undergoes palladium(II)-catalyzed [3,3]-sigmatropic rearrangement to give the corresponding (E)-3-acetoxy-1-propenyl glycine.[125] Addition of diazomethane gives a 1:1 mixture of stereoisomers from which the natural α-(methylenecyclopropyl)glycine (31) was obtained through standard procedures.

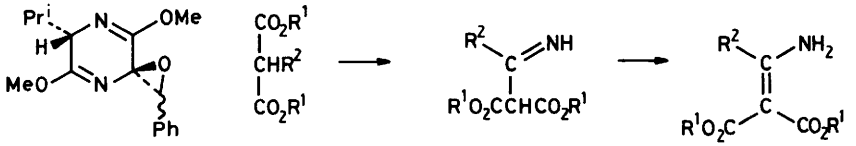
Reaction of 9-allyl- and 9-but-2-enyl-9-borabicyclo[3.3.1]nonane with Schiff bases (S)-(-)-PhCHMeN=CHCO$_2$Bu gives high yields of allylglycines (e.g. 92% yield of the (S)-enantiomer in 92% enantiomeric excess, for allylglycine itself).[127]



Scheme 6



Scheme 7



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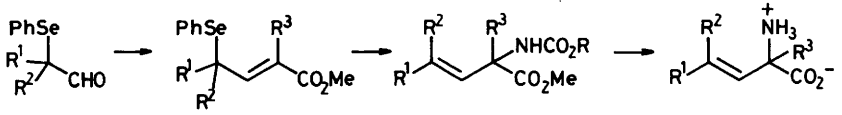
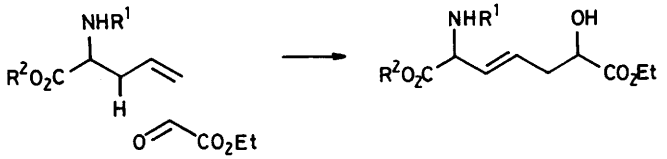
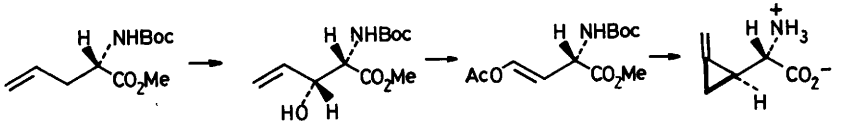


Scheme 8



Scheme 9



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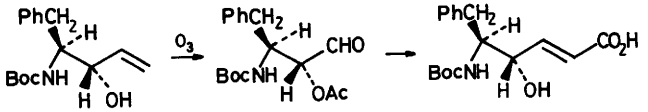
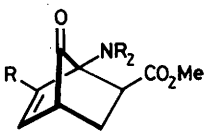


Scheme 10



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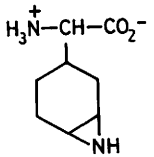

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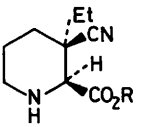

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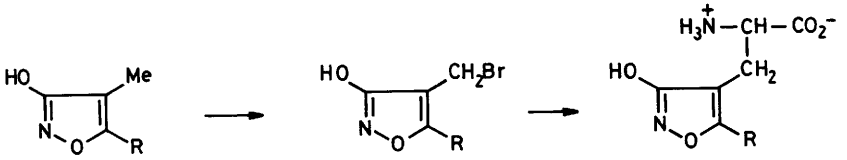

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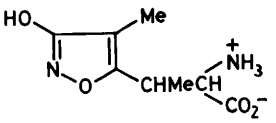


(36)

2-Aminobutadienecarboxylates can be obtained from β-allenyl-α-amino acid esters by a prototropic shift (reaction with ^{t}BuOK during 10 minutes).[128] The products can undergo cycloaddition to give more complex β-amino acids [e.g. (32) with methyl acrylate].

Acetylenic amino acids, an unconventional but common usage for amino acids with carbon-carbon triple bonds in the side chain, are represented by (2R,3R)- or (2S,3S)-2-acylamino-3-phenylhex-5-ynoic acids in the current literature. They were prepared from $HC{\equiv}CCH_2CHPhCH(CO_2Et)_2$ by conventional amination-decarboxylation or modified Curtius rearrangement procedures.[129]

Where the amino and carboxy functional groups are separated by a four-carbon moiety containing unsaturated groupings, then the compound can be variously described (e.g. as a 'keto-vinyl' or 'hydroxyethylidene' isostere of a dipeptide, if such groupings are present in the requisite locations). Synthesis of these novel dipeptide analogues employs stereocontrolled reactions (Scheme 10), starting with Boc-L-phenylalaninal.[130]

4.12 Synthesis of Amino Acids with Aromatic or Heterocyclic Side-chain Groupings.- Modifications of the protein amino acids (phenylalanine is converted into o- and m-hydroxy-L-tyrosines through the involvement of phenylalanine hydroxylase and tyrosine hydroxylase,[131] and 3-cyano-L-tyrosine is obtained from tyrosine via the 3-formyl derivative[132]) are frequently reported in this Section in preceding volumes. Synthesis is often not the main objective in such studies, which are supported by biosynthetic considerations.

Saturated heterocyclic side chains are rarely encountered in natural amino acids but feature occasionally in pharmaceutical studies. (3,4-Iminocyclohexyl)glycine (33) has been prepared from the corresponding cyclohexenylglycine by reaction with INCO.[133] Compounds more closely related to natural amino acids, e.g. the pipecolic acid derivative (34) prepared from L-asparagine, are also represented in the recent literature.[134]

2-Amino-4-(3-pyridyl)butyric acid has been prepared through a Strecker synthesis that starts with 3-(3-pyridyl)propionaldehyde,[135] including α-chymotrypsin resolution (Section 4.15).

A considerable number of ibotenic acid analogues (35) has been prepared from the isoxazoles through side-chain bromination with N-bromosuccinimide, the resulting bromomethyl compound being used to alkylate acetamidomalonate esters in a conventional application of this general method. Where the substituent R of the isoxazole is an ethyl group, bromination places the halogen on the carbon atom adjacent to the isoxazole ring, and use of acetamidomalonate leads to the analogues (36).[136]

4.13 Synthesis of Amino Acids with Side chains Containing Sulphur or Selenium.- This Section regularly depends on cysteine derivatives and near relatives for its existence, and the same applies this year. S-Adenosyl-L-homocysteine can be prepared from L-homocysteine and

5'-deoxyadenosine, employing sodium in liquid ammonia to generate the thiolate anion that adds to the purine.[137] S-(5'-Deoxy-5'-adenosyl)-(±)-2-methylhomocysteine has been prepared similarly;[138] with α-methylmethionine as starting material, sodium in liquid ammonia is used to cleave the methionine into the corresponding thiolate, exploiting the attack at the S - CH_3 bond that is somewhat surprisingly predominant, almost exclusive.

O-Acetylhomoserine sulphydrylase- catalyzed synthesis of L-selenocystine and L-selenohomocysteine from Na_2Se_2 and O-acetylserine and O-acetylhomoserine has been described for potential large-scale implementation.[139] The new selenium-containing amino acid, L-selenodjenkolate, has been prepared from selenocysteine, which in this case was obtained by conventional reductive selenation of β-chloro-L-alanine using Se and $NaBH_4$.[140]

4.14 Amino Acids Synthesised for the First Time.- This 'catch-all' Section again serves to record amino acids newly synthesized by methods that need no special discussion. The reader seeking comprehensive coverage of the title of this Section will need to browse in other parts of Section 4 of this Chapter where new amino acids are occasionally described, often arising incidentally as the outcome of studies of new or modified synthetic methodology.

Amino acid	Reference
3-Aminopyrrolidine-3-carboxylates (from cucurbitin)	141
O-Dipropionylglyceryl NNN-trimethylserine	142
β-(3,4-Diaminophenyl)alanine	43
3-[p-3-(Trifluoromethyl)-3H-diazirin-3-yl] phenylalanine	39
Astatinotyrosine	143
β-(5-Nitro-2-furyl)serine	144

4.15 Synthesis of Labelled Amino Acids.- Examples covered in this Section are grouped on the basis of the label - ^2H, ^3H, ^{11}C, and ^{13}N - and in the order of increasing atomic mass number.

Methods range from conventional general processes to individually designed routes to suit the particular objective. Examples of the former category include a synthesis of L-tryptophan-3,3-$^2H_2$ using the acetylaminomalonate synthesis followed by resolution employing L-acylase,[37] and ^1H - ^2H exchange of the α-proton in DL-α-amino acids in ^2H_2O catalyzed by a salicylaldehyde - formaldehyde copolymer.[145] (4R)- and (4S)-[4-^2H]Homoserine has been prepared, as a mixture of diastereoisomers since the chiral centre at C-2 remained in its initial (RS) state, through straightforward elaboration of (±)-p-$MeOC_6H_4CH(\overset{+}{N}H_3)CH_2CO_2^-$ via (±)-p-$MeOC_6H_4CH(NHBoc)CH_2C^2HO$.[146] 4-[$^2H_2$]-L-Glutamic acid has been obtained in excellent yield by enzymic reductive amination of 4-[$^2H_2$]-2-ketoglutaric acid.[147] The

formation of stereospecifically deuteriated (4R)- and (4S)-[4-$^2H_2$]-L-glutamic acids through separation of the products of $Na(CN)B^2H_3$ reduction of (2RS,4S)- and (2RS,4R)-4-hydroxyglutamic acid derivatives has been shown to involve 75% inversion of configuration in the labelling step.[147]

The well established alkylation of methyl 2-acetamidoacrylate with a Grignard reagent has been used to give fair to good yields, quenching with ^2H_2O giving the α-deuteriated amino acid derivative.[44] The use of CuI in the alkylation step with RMgI as alkylating agent is advocated.[44]

An extensive study in which regiospecifically 2-alkylated 3-deuteriated-1-aminocyclopropane-1-carboxylic acids are obtained starts with 1-deuterio-1,2-dibromoalkanes.[148] Conversion into the cyclopropane-1,1-dicarboylic esters by treatment with di-t-butyl malonate was followed by ester exchange and selective hydrolysis (KOH/MeOH) of the resulting dimethyl ester, leaving the more hindered ester unchanged, and allowing it to be manipulated into an NH_2 group through standard procedures.

3-[^{3}H]-5-(4'-Azobenzenearsonic acid)-L-tyrosine has been prepared from N-Boc-di-iodo-L-tyrosine through reaction with $^3H_2$, substitution with diazotized arsanilic acid, and removal of the Boc group.[149] Meso-2,6-diamino-[3,4,5-$^3H_3$]-1,7-heptanedioic acid is obtained through $^3H_2$/Pd-C-catalyzed dehydrochlorination of 3- or 4-chloro diaminopimelates formed by chlorination of meso-diaminopimelic acid by Cl_2 in conc. HCl.[150]

Several papers have appeared, extending the already substantial literature on synthesis of ^{11}C-labelled amino acids.[151-156] The short half-life of the light carbon isotope calls for rapid procedures covering synthesis, purification, and resolution, let alone rapid use in medical applications where the movement and accumulation of particular amino acids is of interest. Phenylmagnesium bromide treated with $^{11}CO_2$ and reduction gives $Ph^{11}CHO$, employed in the azlactone route with hydrogenation over a chiral Rh(I) catalyst, and resolution, to give 3-^{11}C-L-phenylalanine.[151] The labelled benzaldehyde has also been used in an accelerated Bucherer-Strecker synthesis of 2-^{11}C-DL-phenylglycine (cf. Volume 16 of these Specialist Periodical Reports, p.1).[152] The same group of workers have described a synthesis of labelled methionine, in which S-benzyl-L-cysteine is converted into the $^{11}CH_3$-amino acid through standard sulphur functional-group chemistry after methylation with $^{11}CH_3I$.[153] From what has been mentioned above, ever more speedy chemical operations offer the clinical research worker the best opportunity for time-consuming metabolic studies of these labelled amino acids, as well as merely their accumulation in particular body sites. A preparation time of 50 minutes has been reported[154] for [1-^{11}C]-DL-ornithine and the correspondingly labelled lysine, prepared by the carboxylation of corresponding α-lithioisocyanides by $^{11}CO_2$. Although shorter times have been reported (see earlier Volumes of these Specialist Periodical Reports)

for other syntheses (Strecker methods in particular), problems of side-chain functional groups have to be taken into account in methods based on the construction of parts of the 'glycine' moiety of the target amino acid as the final stage. The same carboxylation route has been used in a synthesis of [1-^{11}C]-DL-proline from α-lithiopyrrolidinyl N-t-butyl formamidine.[155]

[ω-^{13}N]-L-Citrulline, L-[ureido-^{11}C]-L-citrulline, and [carbamyl-^{11}C,^{13}N]carbamyl-L-aspartic acid have been synthesised, employing either ornithine transcarbamylase or aspartic acid transcarbamylase.[156]

4.16 Resolution of DL-Amino Acids.- The crop of recent papers, taken as a whole, amounts to consolidation of established methods. There is some overlap between this and a later section in which analysis of enantiomer mixtures employs separation methods that are essentially the same as those used, or usable, on a preparative scale.

(S)-(-)-Carbamalactic acid, PhNHCOOCHMeCO$_2$H, has been proposed[157] for use in classical diastereoisomeric salt formation resolution of amines and amino acid esters. The same principle is used for the resolution of numerous aryl-substituted phenylglycines by the use of $(+)_{589}$-tartaric acid,[158] and this research group has also reported[159] asymmetric transformation data for the same system to which a carbonyl compound has been added. The principle here is based on reversible Schiff-base formation and the reversible release of the proton at the chiral centre, an equilibrium that is shifted in favour of one enantiomer in the presence of an enantiomer of a chiral catalyst. Another research group has used the same principle combined with the preferential crystallization technique, for asymmetric transformation of DL-p-hydroxyphenylglycine in 95% AcOH at 100°C in the presence of small amounts of salicylaldehyde.[160] Here, preferential crystallization of the toluene-p-sulphonate of one enantiomer by seeding the reaction mixture with the desired enantiomer provides the enantiospecific driving force that is essential to the asymmetric transformation principle.

The preferential crystallization principle continues to accumulate its own literature, partly composed of problem cases as well as a substantial list of examples for which the method is successful. Preferential crystallization of one enantiomer is often inefficient due to the co-crystallization of often substantial amounts of the other isomer, and improvements of a practical nature have been proposed for large-scale resolution of amino acid salts.[161]

Enzymatic methods have been particularly well represented in the recent literature, but no new principles have emerged. Uses of aminoacylases[37,162,163] that leave the D-enantiomer of an N-acylamino acid unchanged by mild hydrolysis include an example of the longest extant laboratory method under this heading, N-chloroacetyl DL-1-aminocyclopropane-1-carboxylic acids[148] were subjected to porcine kidney acylase I-catalyzed hydrolysis and reaction mixtures worked up by standard methods.[162] The same underlying principle is exploited in the catalyzed hydrolysis of DL-phenylthiohydantoins illustrated in the preparation of D-phenyl-

glycine (catalyzed by hydroxypyrimidine hydrase and N-carbamyl-D-amino acid hydrolase),[164] in the use of benzylpenicillinacylase with N-phenylacetyl-DL-α-methyl-α-amino acids,[165] in papain-catalyzed condensation of N-benzyloxycarbonyl-γ-carboxy-DL-glutamic acid with phenylhydrazine (followed by removal of the phenylhydrazide grouping from the L-enantiomer with $FeCl_3$)[166] and extension of this method to all 20 'protein amino acids',[167] and in the production of D-arylglycines in a two-phase liquid system employing immobilized subtilisin for catalyzed esterification of the L-enantiomer.[168] At the other end of the scale, separation of picomole levels of ^{11}C-labelled L-amino acids as their L-aminoacyl-tRNA complexes from the free D-amino acid has been employed[169] in an assay for enantiomeric purity of products from a necessarily rapid synthesis, purification, and resolution procedure (see also refs.151-153). Refs. 135 and 324 describe the use of α-chymotrypsin in resolution of DL-amino acids.

Methods based on physical separations of diastereoisomer mixtures are represented by the flash-chromatographic separation of N-acetyl DL-amino acid L-phenylalanine amides.[170] An aqueous two-phase polymer system based on Dextran 40 - PEG 6000 with serum albumin restricted to the lower phase has been used to resolve DL-tryptophan by counter-current distribution (L-tryptophan is retained in the lower phase).[171] Somewhat similar principles underlie the 'host-guest' approach, in which selective incorporation of one enantiomer into a chiral host molecule is involved. Initially promising results[172] with a chiral Schiff-base polymer that is capable, after complexation to cobalt(II) ions, of hosting D-phenylalanine preferentially from the racemate, have been developed further to give almost 100% stereospecificity. The latter result was obtained[173] for a copolymer prepared from the N-benzyl-D-valine-Co(III) complex of (37) with styrene and divinylbenzene from which the valine was removed to give the 'host'.

Kinetic resolution accompanies the stereoselective reduction of N-hydroxy-DL-amino acids by a chiral thiol in the presence of Fe^{2+} salts.[174] In this fascinating study it is shown that L-amino acids accumulate when (-)-dihydrolipoic acid is the reducing agent, while the D-enantiomers are more rapidly formed when L-cysteine is involved.

A topic of continuing interest, related to the prebiotic predominance of L-amino acids with respect to their enantiomers, concerns a role for chiral radiation in preferential destruction of (or other chemical change to) the D-isomer. The Vester-Ulbricht theory (that natural β-radiation that is inherently chiral behaves in such a way) has been discarded in recent years following the reasoning of Keszthelyi and co-workers.[175] However, recent theoretical work shows that it seems wrong to reason that there is no discrimination between amino acid enantiomers.[176] Clearly, further exploration of these ideas would be useful, and the debate will no doubt continue. There is no better next step than to obtain experimental evidence, and some further papers are cited in the later section of this Chapter (6.1 Racemization) on the unequal outcome, as far as the two enantiomers are concerned, of irradiating amino acids.

5 Physical and Stereochemical Studies of Amino Acids

5.1 Crystal Structures of Amino Acids and Their Derivatives.- The papers under this heading are mainly factual reports, though often there are points of interest that emerge from the specific content of the subject of the study, or more general principles when the results are correlated with other literature reports.

The crystal structures of L-glutamic acid hydriodide,[177] N-acetyl-L-histidinamide,[178] N-benzoyl-DL-alanine ethyl ester,[179] Z-N-acetyl-α,β-dehydrophenylalanine methylamide,[180] N-tritylaziridinecarboxylic acid and N-tritylproline,[181] 2-alkyl-1-aminocyclopropane-1-carboxylic acids,[148] N-acetyl-DL-methionine,[182] L-asparagine monohydrate and L-asparagine + L-aspartic acid monhydrate,[183] and β-amino-γ-hydroxybutyric acid.[184]

A review of a large number of X-ray crystal structures of amino acids[185] from the point of view of minimum contact distances between the non-polar side chains (leucine, isoleucine, valine, and phenylalanine) shows that the preferred interatomic distances in the crystalline state are 0.3-0.5 Å greater than the minimum contact distances.

5.2 Nuclear Magnetic Resonance Spectrometry.- Many physical and stereochemical studies are based on more than one technique, particularly in the spectroscopic area as far as amino acids are concerned. Some cross-referencing between this and succeeding sections is therefore necessary.

Simultaneous appearance of thorough reviews of the n.m.r. of amino acids and peptides is to be noted; one of them[186] covers the literature that appeared mostly within the period 1983-4, while the other[187] is more narrow in its timescale.

^{1}H-N.m.r. studies, excluding the routine uses in support of synthetic work, cover linear relationships that appear to exist between the chemical shift of the amide proton of N-acetyl aspartic acid and temperature.[188] This relationship permits a novel proposal to be made: that this resonance is a useful index for the local temperature within a tissue sample.[188] Other straightforward applications of ^{1}H-n.m.r. form part of an X-ray/n.m.r. study of inter- and intramolecular interactions in N-benzoyl-DL-alanine dithioester[179] and part of a c.d./n.m.r. assignment of absolute configuration to galantinic acid, established[189] to be (2S,4S,5S)-5-amino-2-carboxymethyl-4-hydroxytetrahydropyran (38).

Enantiomeric purity measurements are described[190] for N-acyl-, N-aroyl-, or N-heteroaroyl amino acid methyl esters, based on the separate signals seen for the methyl groups in the presence of the chiral shift reagent Eu(tfc)$_2$. Similar non-equivalence is induced into a range of solutes by methyl esters of N-(3,5-dinitrobenzoyl)-L-amino acids.[191]

The new generation of ^{1}H-n.m.r. techniques is increasingly entering the amino acid field, with consequent benefits in the peptide and protein areas. 2D Double-quantum

coherence values allow the identification of ^{1}H-n.m.r. resonances arising from methyl groups in proteins, for example, and the spectral characteristics associated with methyl groups in alanine, valine, isoleucine, leucine, and threonine have been illustrated.[192] Similar studies (CIDNP - COSY and CIDNP - NOESY) have been reported for photochemically induced dynamic nuclear polarization 2D ^{1}H-n.m.r. spectra of N-acetyl-L-tyrosine and N-acetyl-L-tryptophan.[193]

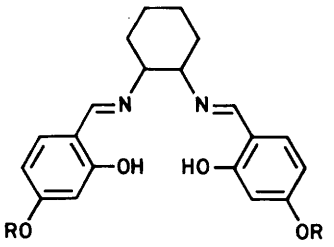
A solution to a simple but frustrating problem, the differentiation of side-chain N-substituted histidines, has been found in n.m.r. spectrometry. Unambiguous differentiation between π- and τ-substitution by a substituent of the general structure RCH_2- is obtained by methylation, followed by nuclear Overhauser effect n.m.r. study. There is an enhanced CH_2 signal for a π-substituent when the adjacent proton between the ring nitrogen atoms is irradiated.[194,195]

As well as accumulating results from several physical methods in tackling problems of structure and dynamics in solution, it is often beneficial to use n.m.r. measurements based on two or more nuclei. ^{1}H- and ^{13}C-N.m.r. spectra of aspartic acid as a function of pH indicate the intramolecular non-bonded interaction of carboxy groups that sets in at higher pH, involving a six-membered ring structure.[196] Vicinal ^{13}C - ^{1}H, ^{15}N - ^{1}H, and ^{13}C - ^{15}N spin coupling constants derived from ^{1}H and ^{13}C n.m.r. spectra for various ionic forms of amino acids, and their ^{15}N isotopomers, have been determined. When reviewed in the light of the potential information in terms of conformational assignments that these can offer, it seems that the ^{13}C - ^{15}N data have relatively little value.[197]

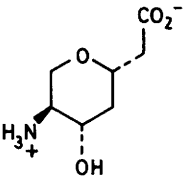
^{2}H-N.m.r. studies of solid amino acids have been interpreted to reveal the re-orientation of deuterons in the $N^{2}H_3$ group of the α-glycine crystal.[198] Similar solid-state dynamics studies of phenylalanine, but including ^{13}C-n.m.r. data as well, reveal that the amino acid is in a state that allows about half the molecules in the crystal to undergo rapid two-fold flips when crystallized from water.[199] The C^{β} - C^{α} bond is the site at which the rotation occurs; the situation is quite different for other methods of crystal formation, and this is clearly opening up a valuable role for n.m.r. studies in solid-state assessment.

^{13}C-N.m.r. chemical-shift values for the α-carbon of an amino acid mainly reflect the electronic shielding of that atom by nearby groupings and has been proposed[200] as a useful parameter for QSAR studies. A straightforward extension of ^{1}H-n.m.r. methodology is seen in the splitting of the resonances of the enantiomeric methylene carbon atoms in 1-aminocyclopropane-1-carboxylic acid, when this amino acid is condensed into a dioxopiperazine (39) with an L-amino acid.[201]

^{17}O-N.m.r. studies have seemed to offer little of distinctive diagnostic value in the past, whether to amino acid studies in particular or relatively complex organic structures in



(37) R = *p* - vinylbenzyl



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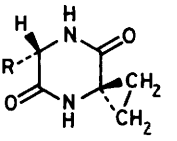

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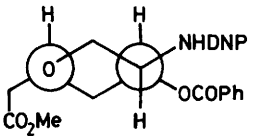

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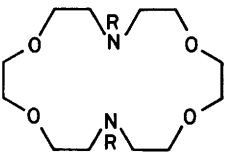
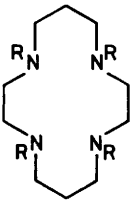


(41) R = $PhCH_2$ or PhCO, also RN in place of O



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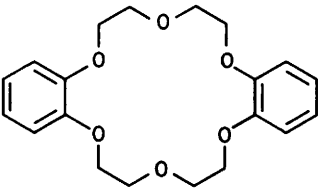

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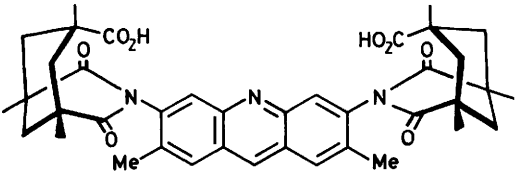


(44)

general. ^{17}O-N.m.r. chemical shifts of the carboxylate-group oxygen atoms of 20 'protein amino acids', together with hydroxyproline, sarcosine, and N,N-dimethylglycine, fall in the range 250 - 257 ppm at low pH but somewhat more downfield (266 - 273 ppm) at neutral pH, with reference to water as an external standard.[202] This study seems a little at odds with a parallel investigation[203] in which the range of chemical shifts in water is found to be identical (249 - 257 ppm) with that reported by Lauterwein et al. for solutions of low pH.[202] Common α-amino acids show ^{17}O-n.m.r. resonances at 234 - 243 ppm in FSO_3H.[203] Comparisons of factors controlling the ^{17}O-n.m.r. chemical shift have been made[204] for cis- and trans-N-acetyl-L-proline and the corresponding N-acetylsarcosine geometrical isomers, taking account of the values for the amino acids themselves. There now seems to be scope for extending the technique to N-acetylamino acid amides and peptides since the reproducibility and resolution of the instrument have been established.

^{19}F-N.m.r. of 3-fluorophenylalanine and 3-fluorotyrosine in bicarbonate buffer reveal a pH-dependent upfield resonance accompanying reversible carbamate formation (NH_2- ⇌ $^-$OCONH-), as well as the resonance for the free amino acid seen in aqueous solutions.[205] This offers a simple method for measuring the equilibrium constant for the condensation.

5.3 Optical Rotatory Dispersion and Circular Dichroism.- A quiet year for these techniques; a representative citation[189] describes the assignment of absolute configuration to (+)-galantinic acid (38; Volume 17 of this Specialist Periodical Report, p.8) based on Kawai's 'DNP - aromatic rule': the positive Cotton effect at ca. 400 nm is consistent with configuration (40).

5.4 Mass Spectrometry.- Discussion of the analytical use of mass spectrometry in conjunction with another technique can be found in the later Section 7: Analytical Methods.

Routine measurements are not covered for this Section, but even the more sophisticated ionization methods are entering into a consolidation-of-existing-knowledge phase. There is continuing interest, of course, in obtaining spectra for the amino acids themselves, avoiding the need to derivatize. Chemical ionization mass spectrometry of amino acids with aromatic side chains, including compounds related to DOPA and tryptophan, gives good spectra with minimum fragmentation when methylamine is used as reactant gas, and analyzing for positive ions.[206] Negative-ion spectra using CCl_4 as reactant gas are also successful.[206] Amino acid methyl esters, converted into oxazolidinones by reaction with 1,3-dichloro-1,1,3,3-tetrafluoroacetone, have provided negative-ion mass spectra by the same method, leading to $M+Cl^-$ amongst other ion - molecule species.[207]

Fast atom bombardment mass spectrometry[208,209] have much to offer for involatile samples, no more so than in the amino acid and peptide field. A distinction between leucine and isoleucine by f.a.b. - tandem mass spectrometry[209] is possible, based on m/z 86 ions;

other examples include the identification of the amino acids in trofopar after derivatization as N-trifluoroacetyl methyl esters [210] and structure assignment to pipecolic acid derivative (2).[17]

Straightforward applications have been described for trimethylsilyl derivatives of N-(1-deoxyhexitol-1-yl)amino acids,[211] $\alpha\beta$- or $\beta\gamma$-unsaturated α-amino acid esters,[212] imino acids (proline and pipecolic acid and their derivatives) in biological fluids at nanomole levels,[213] and assessment of ^{15}N-enrichment in the D-alanine content of bacterial cell walls,[214] for cells grown in $^{15}NH_3$-containing media. In the last-mentioned study,[214] the spectra were measured after conversion of the amino acid into its N-heptafluorobutyroyl D-2-butyl ester.

5.5 Other Physical Studies.- Whereas the preceding Sections would be of most interest to the practising scientist employing amino acids for synthetic and structural studies, there is clearly a wide range of other physical methods that can be applied. The scope of this Section indicates that all available methods are being applied, and in increasing detail. Perhaps this is hardly surprising for a group of organic compounds of such supreme importance in biological contexts in particular, but also in chemical studies.

Spectroscopic and related physical techniques not covered in the preceding sections include ultraviolet Raman spectroscopy of aromatic amino acids (the spectra show considerable dependence on the excitation wavelength),[215] fluorescence spectroscopic study of the binding of tryptophan derivatives to lipid bilayers,[216] infrared spectrometric study of 1-deoxy-1-glycino-D-fructose (the Amadori condensation product of D-glucose with glycine),[217] spectroscopic study of the barrier to rotation about the thioamide bond in N-thionaphthoylsarcosine,[218] dielectric spectra of amino acids in aqueous solutions over the frequency range 1 MHz to 40 GHz,[219] and electron spin resonance spectroscopy of the tyrosyl radical in aqueous solutions.[220] In the last-mentioned study, spectra measured at elevated temperatures (above 60°C) refer to all the possible rotational processes that are conceivable and provide the first complete characterisation of the tyrosyl radical.[220]

Measurements determined with relatively simple apparatus usually based in the teaching laboratory include dissociation constants of amino acids using the ionophoretic technique,[221] volumetric virial coefficients of N-substituted amino acids,[222] enthalpy of interaction measurements for β-alanine with urea in aqueous solution (essentially the same as that for alanine itself in the same system),[223] enthalpies of dilution of N-acetyl-L-prolinamide with equimolal solutions of other N-acetylamino acid amides,[224] and of solutions of these solutes in NN-dimethylformamide compared with data for aqueous solutions.[225] These properties reflect all facets of solvent - solute and solute - solute interactions of particular interest. The heats of dilution data for aqueous solutions of D-alanine amides containing various amounts of the L-enantiomer reveal chiral recognition arising through diastereoisomeric pairwise

interactions.[226]

Some simple physical properties that include important biological roles, at least in principle, have been reported. Proline is suggested to be a natural cryoprotectant,[227] preserving membrane structure and function as far as Ca^{2+} transport is concerned, under freezing conditions. Seleno-DL-methionine offers some protection for human platelets against freezing injury.[228] The sites at which these amino acids exert their effect may not be identifiable without knowledge of the transport properties of the amino acids themselves, as well as of other solutes. Transport properties of amino acids is a long-standing research topic, and recent studies cover the transport of β-alanine and non-protein α-amino acids across brush-border membranes of rabbit ileum.[229] These studies frequently employ labelled amino acids, and the uptake of [^{3}H]-L-tryptophan by rabbit forebrain synaptosomes[230] is an example of this. In this study, the kinetics of the process were of particular interest, with the demonstration that extracellular sodium ions reduce the rate of uptake.[230] In model systems of potential biological relevance, polyamine and polyamide macrocycles (41) and (42) and polyethers (43) are excellent cation carriers for the transport of amino acid ester salts.[231] In the same category, the compound (44) shows a remarkable ability for the extraction of phenylalanine, tryptophan, and o-methyltyrosine into chloroform from water.[232] There is thought to be three-point binding involving particularly the acridine moiety in a stacking-type interaction with the aromatic groupings of these amino acids.

Interactions between amino acid amides and polyribonucleotides are also a long-standing subject of research, and can be detected by measuring melting temperatures of such systems as a function of concentration.[233]

A series of N-acyl-DL-amino acids has been studied by differential scanning calorimetry to add to knowledge of structures known to form solid racemic compounds.[234] Other points of interest concerning amino acids and their derivatives in the condensed state arise for N-(n-dodecanoyl)-L-alanine (adopts the cholesteric mesophase liquid crystalline state)[235] and a fascinating observation[236] that a structural correlation exists between an etched crystal surface (either enantiopolar glycine or enantiomorphic L-asparagine hydrate) and the etchant. This provides a new means of manual sorting of enantiomorphous crystals and also a new method for assigning absolute configuration to a crystalline enantiomer.

5.6 Molecular Orbital Calculations.- Apart from calculations for atomic charges at all locations in amino acids and peptides,[237] most current papers address conformational problems. Representative citations from major research groups deal with N-acetyl dialkylglycine N-methylamides[238] and energy differences between low-energy conformers of N-acetyl glycine N-methylamide.[239]

The very different aspect of energy involved in calculations of parity-violating weak

neutral current perturbation of amino acid electronic energies has an important implication: the energy shifts that are caused are consistently favouring the L-enantiomer of a racemic pair.[240]

6 Chemical Studies of Amino Acids

6.1 Racemization of Amino Acids.- Aspects of resolution discussed in Section 4.15 are based on the separation of one enantiomer from a solution of a racemate in which the other enantiomer is being caused to racemize. The underlying chemistry has also been used in a study optimizing the racemization of amino acid esters in organic solvents by aromatic aldehydes as catalysts. The aldehyde may be a solute or bonded to a solid phase.[241]

A continuing research topic has been christened 'radioracemization' - the search for experimental proof that destruction of one enantiomer of a DL-amino acid by γ-radiolysis is more rapid than for the other. γ-Radiolysis of L-leucine and its hydrochloride, in aqueous solution or adsorbed on clays (kaolin or bentonite), has been continued so that between 2% and 89% of the amino acid is 'destroyed', and it is accompanied by small levels of racemization. L-Leucine in aqueous solution is least stable to radiolysis and radioracemization and the amino acid in the solid state is most stable to the conditions, with the amino acid adsorbed on clays occupying an intermediate position.[242; see also 348]

6.2 General Reactions of Amino Acids.- This Section is followed by one entitled 'Specific Reactions of Amino Acids', in which reactions of the amino acid side chain, in isolation or in concert with the amino and carboxy groups, are covered. Therefore, this Section is essentially concerned with reactions of amino acids irrespective of the side chain. This coverage falls broadly into three headings: reactions at the amino group, reactions at the carboxy group, reactions involving both carboxy and amino groups.

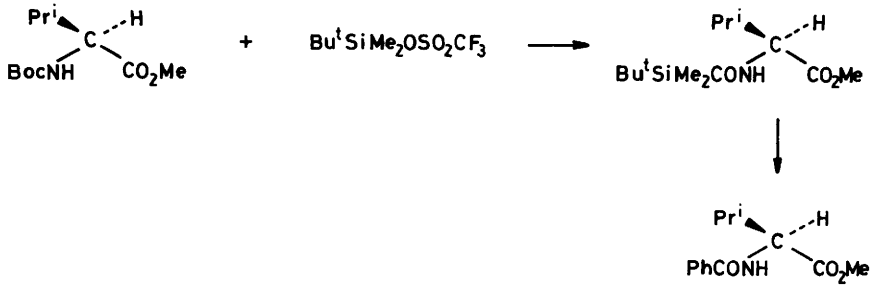
'Reactions at the amino group' and the following section largely exclude the synthesis of protected derivatives for use in peptide synthesis, unless there is any unusual interest. N-Boc-amino acids, for which a representative Organic Syntheses recipe now exists[243] (for Boc-L-phenylalanine) employing Boc-anhydride, can be converted into their N-ethyl analogues through treatment with ^{t}BuLi and then $Et_3O^{+}BF_4^{-}$.[244] A second Boc group can be introduced at the nitrogen atom of a Boc amino acid, if the carboxy group is esterified, using Boc anhydride in MeCN containing the non-nucleophilic base 4-dimethylaminopyridine.[245] The esterifying grouping used in this work was the benzyl group, permitting its selective removal from the product. A convenient preparation of carbamates of α-amino acids[246] involves reaction with a chloroformate in refluxing ethyl acetate. An interesting preparation of a trimethylsilyl carbamate, an intermediate that can be converted into any other alkyl

carbamate through reaction with an alkyl halide, has been described.[247] The general scheme (Scheme 11) allows the conversion of an N-Boc-L-amino acid ester into its N-benzyloxycarbonyl analogue without racemization, in 85% yield in the case of L-valine methyl ester. It is also compatible with other protection regimes, for example side-chain unsaturation and the protection of hydroxy groups as acetonides in multifunctional amino acids (45); see also ref 125.

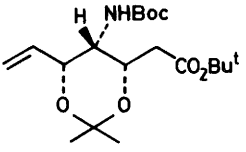
A group of papers that cover problems associated with standard analytical procedures contributes to ever more reliable assays. N-Methylation and NN-dimethylation accompany methyl ester formation with amino acids, when diazomethane is used as part of a procedure for the conversion of amino acids into volatile derivatives for g.l.c. analysis.[248] Metal ions interfere in reactions of the amino group with the fluorogens dansyl chloride, o-phthaldialdehyde, fluorescamine, but especially 4-fluoro-7-nitrobenzo-2-oxa-1,3-diazole.[249] The net result, the suppression of the fluorescence to variable extents, can be overcome if EDTA is added to the reaction mixtures. Surfactants also act to inhibit various colour reactions that are commonly used in amino acid analysis, and the effect is particularly noticeable with non-ionic surfactants.[250] Further attention has been given to the mechanism of the o-phthaldialdehyde procedure that has become a popular choice for pre- and post-column h.p.l.c. analysis of amino acids. The procedure involves a thiol as an essential component and this is shown to be incorporated into the eventual product, a 2-*mercapto*-isoindole, at a final stage. The initially formed imine may not be the only intermediate, as shown by detailed kinetics measurements and their interpretation.[251] It is believed that addition of the thiol to the imine to give an α-alkylaminobenzyl sulphide is followed by isoindole formation.[251,252]

Fluorescent products formed between some of the protein amino acids and 12-oxo-cis-9-octadecenoic acid have been characterized.[253] The aliphatic acid is otherwise known as 12-keto-oleic acid, a by-product of lipid peroxidation; apart from glycine, only the basic amino acids yield strong fluorescence, which is diminished by N-acetylation and inhibited by the addition of thiols. Methyl 12-keto-oleate reacts with amino acids to give 8-(N-substituted 4,5-dihydro-4-oxo-5-hexyl-5-hydroxy-2-pyrrolyl)octanoic acid methyl esters (46).[253] Other results of interest concerning reactions between amino acids and aliphatic compounds that accompany them in vivo have been reported: for melanoidins formed in the Maillard reaction between glucose and glycine, and their separation over a strong anion-exchange resin,[254] and for the 'eneaminol' (47), the initially formed adduct between D-xylose and glycine when reacted in equimolar amounts in water at 68°C.[255] The eneaminol is clearly formed between the reactants in a 2:1 ratio, and the use of equimolar amounts may simply have been the way that the experiment was actually carried out, rather than the outcome of a series of experiments designed to consign an optimized procedure to the literature.

Other reactions at the amino group include a careful study of the well-known reaction



Scheme 11



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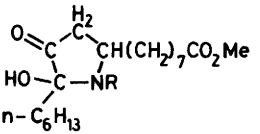

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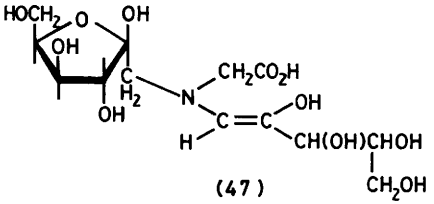

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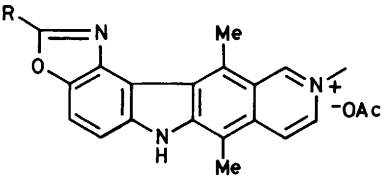

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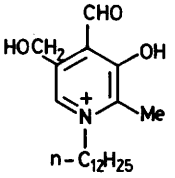

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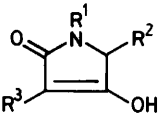

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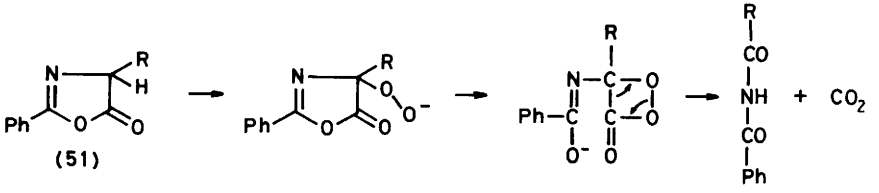


(51)

Scheme 12

with nitrous acid in the presence of halide ions, leading to L-α-halogenoalkanoic acid esters from the corresponding α-amino acid esters,[256] peroxidase-catalyzed reaction with N-2-methyl-9-hydroxyellipticinium acetate leading to condensed oxazoles (48),[257] and correcting earlier structures assigned to the product, and Schiff-base formation with the pyridoxal 5'-phosphate model (49) and transamination reactions involving the product.[258]

Cleavage of promising N-protecting groups has been the subject of mechanistic study for diphenylphosphorylamino acids ($MeOH/H_3O^+$)[259] and N-(1-benzotriazolylcarbonyl)amino acid esters (Et_3N-catalyzed alkanolysis, but only in moderate yield).[260]

The already voluminous literature on oxidative degradation of amino acids is further augmented by kinetic studies using Chloramine-T,[261] phenyliodosyl acetate,[262] ClO_2,[263] bromate,[264] N-bromosuccinimide,[265] and N-bromoacetamide.[266] Catalysis of the oxidative decarboxylation by carboxylate anions is a curious observation arising from the last-mentioned study.[266]

N-Protected amino acids can be converted into their diphenylmethyl esters using benzophenone hydrazone, iodine, and phenyliodosyl acetate.[267] Rapid esterification of amino acids through temporary N-protection by condensation with ethyl acetoacetate, treatment with an alkyl chloride, and N-deprotection with toluene-p-sulphonic acid gives amino acid ester toluene-p-sulphonates in 75-89% yields.[268] Long-chain alkyl esters of amino acids have been obtained by reaction with the alkanol and $MeSO_3H$, using the alkanol ($C_{18}H_{37}OH$ was used in this study)as solvent as well as reactant.[269] A reaction mixture containing an amino acid, an alkyl toluene-p-sulphonate, and the corresponding alkanol proceeds to the amino acid ester utilizing the alkanol, not by transesterification of the sulphonate ester.[270]

Isopropenyl chloroformate has been proposed[271] for the preparation of carbonates of N-protected amino acids for use as 'active esters' in peptide synthesis. An interesting, though negative, result has been reported for cyanoacetylene, thought to be a peptide-forming agent relevant to ideas of prebiotic protein synthesis: it does not react with N-protected amino acids.[272] Conversion of N-trifluoroacetylamino acids into primary amides has been accomplished by condensation with hydroxylamine using dicyclohexylcarbodi-imide, followed by reaction with NH_3.[273]

Disproportionation of phosphinic - carboxylic anhydrides of N-protected amino acids has been subjected to kinetic study.[274] Other studies dealing with reactions of carboxy-group derivatives of amino acids deal with uses of N-trifluoroacetylamino acid chlorides in Friedel-Crafts acylation reactions leading to aryl N-trifluoroacetylaminoalkyl ketones,[275] formation of α'-amino-αβ-ynones via α-amino acid isoxazolidides,[276] and continuing studies of enantioselective hydrolysis of DL-amino acid p-nitrophenyl esters by micelles containing L-histidine.[277] A closely related study[278] with the same objective employs N-dodecanoyl-

DL-phenylalanine p-nitrophenyl ester as substrate and micelles containing N-benzyloxycarbonyl-L-phenylalanyl-L-histidyl -L-leucine as chiral catalyst.

Schiff bases formed between pivalaldehyde and an α-amino acid N-methylamide give trans-imidazolidin-4-ones (11) when cyclized with benzoyl chloride but cis isomers when benzoic anhydride is used.[279] Other studies in which both amino and carboxy functions of amino acids are involved include the formation of pyrrolidine-2,5-diones (50) by cyclization of N-acylalanine and glycine esters with NaH in DMF, and their alkylation and acylation behaviour.[280] Whereas acylation occurs exclusively on oxygen, alkylation leads to a mixture of O- and C-alkylated products. Oxazolin-5-ones (51) formed from N-acylamino acids undergo base-catalyzed decarboxylation after adding O_2, leading to diacylamines (Scheme 12).[281] A new synthesis of oxazolidin-2,5-diones ('amino acid N-carboxy anhydrides' has been reported[282] in which N-Boc-amino acids are reacted with $^tBuMe_2SiCl$ and the resulting silylester is reacted with oxalyl chloride.

Reactions of potential relevance to prebiotic condensation reactions of amino acids are discussed in a substantial set of Symposium papers, two of which are typical: thermal copolymerization of a mixture of 18 amino acids gives readily reproducible mixtures of peptides,[283] and the reactivity of amino and carboxy functional groups of amino acids can be assessed to account for preferred combinations between constituents in amino acid mixtures.[284] Acid catalysis of gas-phase cyclization of α-amino acids has been assessed,[285] and a co-operation of amino and carboxy groups is seen in the hydroxyl radical-induced decarboxylation of amino acids in alkaline solutions.[286] The radical is believed to add to the unprotonated NH_2 group in this system and thereby trigger off the loss of CO_2 and simultaneously involve water in the process.

1:1 Complexes formed between 18-crown-6 or dibenzo-18-crown-6 ethers with amino acids or their K^+, Ca^{2+}, or Na^+ salts have been described.[287]

6.3 Specific Reactions of Amino Acids.- Amino acids with side-chain hydroxy groups are again the subject of several recent papers covering reactions involving one or more functional groups. Sulphate ester formation is quantitative using H_2SO_4 in DMF with dicyclohexylcarbodi-imide as condensing agent.[288] A re-investigation of the oxidative degradation of amino acids by Chloramine-T concentrates on nitrile formation from threonine.[289]

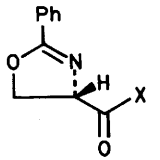
Base-induced $C^\alpha - H$ and $C^\alpha - C^\beta$ bond cleavages in Λ-and Δ-bis{N-salicylidene-(S)-O-acylthreoninato}cobaltate(III) have been compared, revealing the former to be 3 times faster,[290] and competing β-elimination has been assessed.[291,292] 2-Iminopropionate esters formed in this way from O-acetyl and O-alkanesulphonyl serine esters can undergo base-catalyzed addition to aldehydes to reform the γ-hydroxyamino acid ester.[292] During attempted conversion of N-trifluoroacetyl serine and threonine into their trimethylsilyl esters

by reaction with N,O-bis(trimethylsilyl)trifluoroacetamide, a straightforward process for other common amino acids, condensation to form the corresponding 2-trifluoromethyloxazolines, was observed.[293] The 2-phenyloxazoline derived from L-serine methyl ester reacts with 2-alkyl-2-lithio-1,3-dithians to give (52), from which corresponding chiral aminoketones may be obtained without racemization by reductive desulphurization.[294]

Enantiomerically pure N-Boc- or -benzyloxycarbonyl β-lactams (53) formed by the use of Mitsunobu reagents with the corresponding L-serine derivatives are susceptible to ring opening by powerful nucleophiles (e.g. methoxide ion) to give the β-substituted alanines.[295] β-Lactams formed by annelation of Schiff bases can involve D-threonine as starting material; the threonine-derived Schiff base (Scheme 13) gives almost 100% optically pure β-lactams.[296]

Some of the studies of serine derivatives described in the preceding paragraphs have also included cysteine analogues with similar results - the conversion of cysteine into its S-sulphonate[288] and β-elimination of methanethiol from S-methylcysteine co-ordinated to cobalt(III)[292] are accomplished in the ways described for the oxygen analogues. Another β-elimination study[297] employing S-benzyl-DL-cysteine describes the use of N-dodecylpyridoxal micelles for the purpose. A variety of reactions of the thiol group of cysteine are represented in the recent literature and need only brief description: formation of the thiolsulphonate RSH → RS-N=O[298] → $RSSO_2Ph$;[299] reduction of cystine thiolsulphinates to give unsymmetrical cystines and lanthionines, using tris(dialkylamino)phosphines;[300] formation of the methoxycarbonylsulphenyl derivative RSH + $MeO_2CSCl \rightarrow MeO_2CSSR$ that yields thiolcysteine RSS^-K^+ with KSH;[301] formation of thiocarbamoyl and carbamoyl derivatives from N-benzoylcysteine ethyl ester RSH + R-N=C=S → RNHCSSR and RSH + PhN=C=O → PhNHCOSR, respectively (but benzoylcysteine fails to react with PhNCO);[302] formation of N-Boc- or N-Bpoc-N-methyl-4-aminobutanoyl derivatives RSH → Boc- or Bpoc-$NMe(CH_2)_3COSR$, the group being removeable by trifluoroacetic acid;[303] and preparation of N-Boc-{S-(3-nitro-2-pyridinesulphenyl)}cysteine N-hydroxysuccinimide ester as a heterobifunctional crosslinking agent for linking cysteine peptides to bovine serum albumin.[304]

S-Alkylation of cysteine derivatives for different purposes has been reported in a synthesis of a compound related to leukotriene E_4: $Me(CH_2)_{11}C{\equiv}CCH\{O(CH_2)_3CO_2R\}_2$ is added to $CF_3CONHCH(CH_2SH)CO_2Me$;[305] in preparations of chiral phosphines starting with L-cysteine, L-methionine, or D-penicillamine and subjecting them to S-alkylation, N-methylation (formaldehyde/H_2/Pd-C), and carboxy-group reduction to the primary alkanol ($LiAlH_4$);[306] and a conversion of S-t-butylhomocysteine into methionine through S-methylation (MeI and $AgClO_4$) and storage of the sulphonium salt at 40°C during 2 hours.[307] In the last-mentioned study methylation by excess MeI in aqueous emulsion during 2 hours converted the S-t-butyl compound into the S-dimethylsulphonium salt.



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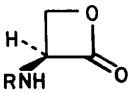

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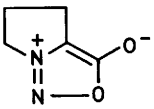

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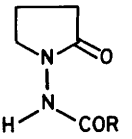

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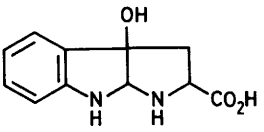

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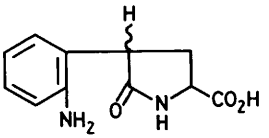

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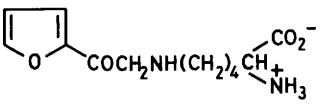

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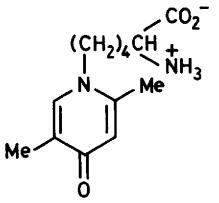

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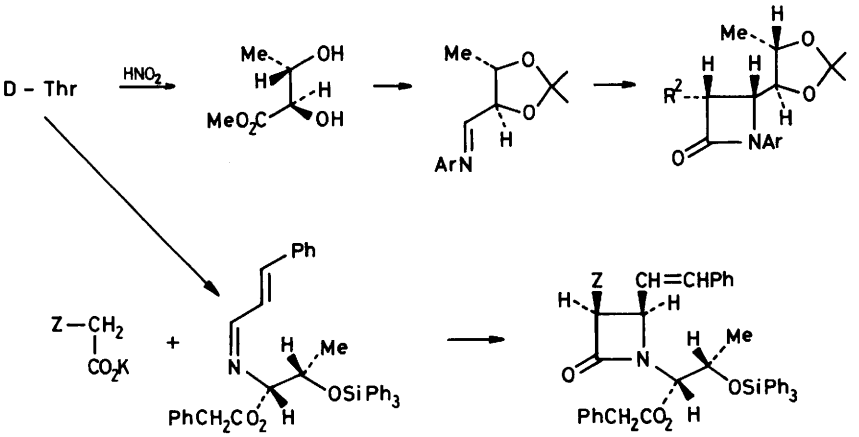

Scheme 13

Pyrolysis of solid L-cystine and its three stereoisomers either at 150°C or at 230°C gives a mixture of NH_3, H_2O, H_2S, and CO_2.[308]

Asymmetric oxidation of methionine derivatives to give the sulphoxide has been accomplished using t-butyl hydroperoxide in the presence of $(Me_2CHO)_4Ti$ - (+)-diethyl tartrate.[309]

Studies involving proline and other imino acids deal with the formation of N-nitroso-L-proline ethyl ester in millet inoculated with Fusarium moniliforme, a common fungus, when nitrite is present in the growth medium,[310] the formation of N-acylazaprolines from proline via a mesoionic derivative (54) → (55),[311] and a related cycloaddition of N-(2-pyridinecarbonyl) proline with 2-chloroacrylonitrile in acetic anhydride (which functions both as solvent and as reagent for forming a mesoionic intermediate from the proline derivative).[312] A surprising but useful oxidative cleavage of nopaline, a member of the 'opine' family discussed on p.2 of this Chapter (refs. 18,30-32), has been described that gives the two constituent amino acids L-arginine and D-glutamic acid without racemization and thereby allows absolute configuration to be assigned.[313] For no obvious reason, the normally straightforward method in this series for assigning absolute configuration - the reaction of L-arginine with enantiomers of 2-chloropentanedioic acid and comparison of products with the natural compound - failed.[313] Aziridine carboxylic acid has been converted, via its potassium salt, into series of esters and amides.[314]

1-Aminocyclopropanecarboxylic acid (whose synthesis in grapefruit tissue is inhibited by ethylene, which enhances the accumulation of its N-malonyl derivative and thereby reduces further the availability of 1-aminocyclopropanecarboxylic acid[315]) breaks down to give not only the well-known products ethylene and CO_2 but also CN^-.[316] Fortunately the cyanide does not accumulate but appears in biosynthesised asparagine. The sequential single-electron transfer pathway for ethylene biosynthesis is given further support through this novel finding.

By their nature, alkyl side chains are not susceptible to selective attack while the amino and carboxy groups are protected only by the relatively labile groups that are used in peptide synthesis. However, N-benzoylvaline methyl ester has been used in studies of free-radical halogenation,[317] with the finding that SO_2Cl_2 and N-bromosuccinimide show different regioselectivity. The stable reagent 4-formyl-2-methyl-1,3,4-thiadiazolin-5-thione converts N-hydroxyglycine into its N-formyl derivative (hadacin, an anti-tumour antibiotic) in 83% yield.[318]

A worrying finding, that 5-phenyl-2-pyridinamine is mutagenic,[319] has to be coupled with the fact that this pyrolysis product of phenylalanine is present in broiled sardines. Heteroaromatic amino acids are represented by tryptophan reactions: oxidative modifications to peptides carrying tryptophan at the N-terminus lead to the generation of its 3-anilinopyrrolidin-2-one derivative that is not susceptible to Edman degradation;[320] generation of light accompanies chlorination of the tryptophan indole moiety by the myeloperoxidase - H_2O_2 - Cl^-

or HOCl or taurine chloramine systems;[321] formation of strongly fluorescent derivatives ($\lambda_{exc.}$ 305 nm, $\lambda_{emiss.}$ 455 nm in dil. HCl) by reaction with chloroacetaldehyde;[322] β-cleavage by phenyliodosyl acetate in methanolic KOH to give 3-methoxymethyl-3H-indole through initial addition of C_6H_5IO to the indole nitrogen atom;[323] α-chymotrypsin-catalyzed esterification of N-acetyl-L-tryptophan and N-acetyl-L-tyrosine in ethanol containing less than 10% of water;[324] and photo-oxidative conversion into 3-(2-aminophenyl)-2-pyrrolidone-5-carboxylates and kynurenine (56) → (57).[325]

Strong acid treatment of α-amino-δ-hydroxyvaleric acid leads to the formation of proline and several unidentified products, presumed to be lactones.[326]

Reactions of the acidic amino acids reported in the recent literature are mostly relatively routine: formation of α-esters of γ-methyl N-benzyloxycarbonyl-L-glutamate;[327] N-trimethylsilylation of pyroglutamic acid with Me_3SiCl allows N-p-nitrobenzylation to be accomplished (but at 150°C);[328] decarboxylation of γ-carboxyglutamic acid by guanidine, a model for the possibility that arginine residues in γ-carboxyglutamic acid-containing proteins, may control the number and position of such residues.[329] A surprising result has emerged from attempted transamination of β-fluoroaspartic acid using pyridoxal 5'-phosphate, where dehydrofluorination occurred instead;[330] the possibility of carboxy-group assistance needs to be excluded by comparative studies with other β-fluoro-α-amino acids.

The basic amino acids are represented by lysine (further studies by Tyihak's group on the mechanism of N^{ϵ}-formylation by condensation with formaldehyde,[331] oxidative conversion into α-ketoglutaric acid catalyzed by saccharopine dehydrogenase and involving the NAD to NAD(H) reaction[332]), hydroxylysine [oxidation by HIO_4 to glutamic acid via the semi-aldehyde, but to Δ^1-pyrroline-5-carboxylic acid by $NaIO_4$ in alkaline conditions,[333] and N-protected α,ω-di-amino acids (RuO_4 oxidation to give the protected α-amino acid ω-amide[334])]. Controversy surrounding the identification of canavanine in alfalfa extracts was initiated some years ago[335] on the basis that the colour reaction on cellulose t.l.c. with pentacyanoammonioferrate was due to histidine instead; it has swung back to refutation in favour of the initial claim.[336] It seems that there is a substantial quantity (8 - 20 g Kg^{-1}) of the toxic amino acid in this plant.[336]

6.4 Non-enzymic Models of Biochemical Processes involving Amino Acids.- Some of the preceding Sections contain topics that could have been located here, too, but their major interest has appeared to lie in the techniques used. The binding of common amino acids to ^{14}C-thymine seems to be enhanced by γ-irradiation[337] and may involve a stacking interaction in the case of aromatic and heteroaromatic amino acids, as seen for tryptophan with uracil by hypochromic shifts and fluorescence characteristics.[338] Effects of glycine, β-alanine, and γ-aminobutyric acid[339] or glycine and glutamine[340] or amino acid amides[233] on the

stability of the DNA double helix have been determined by melting behaviour[233,340] or by c.d. measurements.[340]

6.5 Effects of Electromagnetic Radiation on Amino Acids.- Aromatic amino acids have been treated to vigorous irradiation: pulse radiolysis of 5- and 2,5-cysteinylDOPAs in aqueous solutions containing azide ions causing sequential semiquinone and quinone-imine formation,[341] and u.v. irradiation of aqueous solutions of phenylalanine, tyrosine, and tryptophan in the presence of nitrite or nitrate ions gives 'uncharacterized mutagens'[342] (a quite unacceptable piece of preliminary publication in its lack of information on its important claim).

Milder processes are involved in a study of fluorescence decay kinetics of L-tyrosine as a function of pH [343] and similar studies of homotryptophan.[344] In the latter study, the data were almost identical with those for tryptophan, bringing into question interpretations of the fluorescence behaviour of tryptophan based on assignment of lifetimes to structural states that involve interactions between the heteroaromatic moiety and ionic forms of the aliphatic moiety. A new method for fluorescence decay studies of tryptophan employs synchrotron radiation for pulsed excitation and photon-counting for decay kinetics.[345] More vigorous processes are described for photolysis of tryptophan under aerobic and anaerobic conditions at various pH values of solutions,[346] and an interesting finding that tryptophan radicals formed under irradiation can protect other species in the same environment - in other words, can act as endogenous antioxidants in vivo.[347]

An earlier Section, 6.1, refers to radioracemization of amino acids, a process that does not occur with amino acids crystallized from water by their γ-irradiation in the solid state but does occur for sublimed enantiomers of leucine.[348] These greatly differing quantum yields for decarboxylation through irradiation by non-polarized γ-radiation (D $\sim$ 2L $\approx$ DL) have been reported by a research group that has been active in the field for some time and adds another curious result that defeats explanation. It is significant that three papers reviewed in different parts of this Chapter[199,242,348] describe differing behaviour of solid amino acids as a function of their solid state; with the ever closer approach of chemistry and electrical properties of the solid state, the late 1980's and beyond are going to provide rich rewards to research workers who show the appropriate vigilance in these topic areas.

7 Analytical Methods

7.1 Gas-Liquid Chromatography.- The preparation of volatile derivatives introduces specific problems, and useful efforts continue to be made to eliminate sources of artefacts in these preparations. Heptafluorobutyroylation of arginine isobutyl ester, using a mixture of heptafluorobutyric acid and its anhydride, represents the standard technique of preparing the

most commonly used N and C derivatives. However, the area of the arginine peak in g.l.c. was less than expected, and two spurious peaks were seen.[349] The same workers[350] have described removal of glucose from plasma samples prior to g.l.c. analysis, by its enzyme-catalyzed conversion into glucose-6-phosphate. The same derivatives have been used in analysis of γ-carboxyglutamic acid,[351] and [1-^{13}C]leucine,[352] and the isopropyl ester analogue,[353] n-butyl ester analogue,[354] or ester analogue with (+)-2-butanol,[214] and N-trifluoroacetyl[354] and other perfluoroacyl[355] analogues. Pentafluorobenzoyl amino acid di-n-butylamides have been used for analysis of taurine in plasma at picomole levels [356] and similar applications.[357] Silylation procedures continue to be used occasionally, for analysis of asparagine and glutamine (dimethylsilylation after N- or O-t-butylation) [358] and analysis of selenomethionine.[359]

Mass spectrometric detection and identification of separated components has been used in a number of cases.[214,352,357,360]

Maillard reaction products formed between lysine and fructose, or lysine and lactulose, give hydrolysis products furosine (58) and pyridosine (59), according to g.l.c. analysis.[361]

Enantiomeric purity of amino acids can also be assessed by g.l.c., through methods that are now standard. Either the volatile derivative of the amino acid is separated into its enantiomers over a chiral stationary phase (N-pivaloylproline methyl ester over Chirasil-Val,[362] and a wide range of examples over chiral polysiloxanes of various types[363]) or the analysis sample is converted into a diastereoisomer mixture[364-366] (e.g. reaction of the D- and L-amino acid ester mixture into the N-trifluoroacetyl-L-prolyl derivative[366]). As was mentioned earlier in this Section, introduction of artefacts that cast doubt on the analytical accuracy can be anticipated in any derivatization procedure, and the inconsistent results reported in the last-mentioned study have been ascribed to partial racemization of the reagent, N-trifluoroacetyl-L-prolyl chloride, used in this case.[366] The conclusion is surprising, because the reagent would not be expected to undergo racemization even in the presence of triethylamine that is required for the process; however, the inconsistent results only arise at low D:L ratios (below 1:10).

7.2 Ion-Exchange Chromatography.- The small scope of this Section does not relate to the volume of data that is collected regularly under this heading, through a technique that is undergoing continuous instrumental development, merely that there is little of substantial chemical interest in the recent literature. Overlap with the later h.p.l.c. section should be borne in mind by readers seeking representative coverage.

General methodology is described using h.p.l.c. instrumentation and ninhydrin colorimetry;[367] other papers deal with specific analyses (di-aminopimelic acid reaching 2 nanomole levels [368] and glutamic acid - glutamine ratios with norleucine as internal standard [369]). A group of papers share a common interest in cysteine derivatives and near relatives: h.p.l.c.

ion exchange for homocysteine[370] and other routine studies (aminoethyl cysteine[371] and S-aminoethyl-3-mercaptolactic acid [372]) together with a study of the estimation of cysteine + cystine in proteins as 'cysteinoic acid' formed by hydrolysis of proteins in 2% dimethyl sulphoxide - 6M HCl.[373] The last-mentioned study follows earlier suggestions [374] and refers to the formation of cysteic acid; accompanying oxidation of tyrosine, serine, and methionine whether within the protein or after hydrolysis is avoided by adding phenol to the hydrolysis cocktail.

7.3 Thin-Layer Chromatography.- As for the preceding Section, small scope for this Section does not mean that the technique is in any way declining in its use.

Attention has been given to 2-dimensional t.l.c. identification of N^{π}-methylhistidine, since this runs close to abnormal metabolites present in physiological samples.[375] Estimation of free proline in mixtures has been based on isatin colour formation and estimation at 608 nm of the concentration from absorption data, for samples extracted from paper chromatograms.[376] T.l.c. of dansylamino acids on polyamide plates has been given thorough study,[377] and further data have also been secured for resolution of DL-amino acids by ligand-exchange chiral t.l.c. analysis.[378] The principle behind the latter study has a long history, since cellulose itself in the course of conventional paper chromatography and t.l.c. has the ability to separate enantiomeric pairs, but the newer ligand-exchange resolution procedure appears to offer the necessary flexibility to optimise particular analytical applications.

7.4 High-Performance Liquid Chromatography.- In contrast with the relatively static nature of the stage reached for g.l.c., ion exchange, and t.l.c. methods, there is still much development occurring in h.p.l.c. methodology and competition between the various alternatives in amino acid analysis. Six standard methods for h.p.l.c. analysis of amino acids have been compared,[379] and variables that influence the reliability of phenylalanine h.p.l.c. assays, in terms of the handling of samples prior to analysis, have been discussed.[380]

Procedures for amino acids in general (h.p.l.c. ion exchange,[381] isocratic elution employing aqueous copper(II) alkanesulphonates as solvent) and amino acids in particular are briefly cited here. NN-Dimethylglycine can be determined by h.p.l.c. ion exchange and specific detection using dimethylglycine dehydrogenase,[383] cysteine by amperometric detection at less than 4 picomole levels,[384] and S-adenosylmethionine and S-adenosylhomomethionine[385] tryptophan (either electrochemical[386] or fluorescence[387] detection), DOPA, and its m- and p-O-methyl derivatives (electrochemical detection[388]) and related catechol-based amino acids [389] have also been studied using standard methods. Aminohydroxyphenylalanine (a constituent of phaeomelanin),[390] [^{10}B]-p-boronophenylalanine,[391] O-phospho-L-serine, L-threonine, and L-tyrosine [392] and other phospho-amino acids[393] provide examples of specialized research interests.

Resolution based on the ligand-exchange principle employing an aqueous solution of copper(II) L-prolinate as stationary phase has been applied to aromatic amino acids,[394] [^{11}C]-DL-leucine and [^{11}C]-DL-tryptophan. The earlier Section 4.14 has included the point to be made concerning the very short half-life of the ^{11}C isotope, and preparation together with h.p.l.c. resolution took no more than 55 - 60 minutes for these labelled L-amino acids.[395] The classical chiral stationary-phase principle applied[396] to the separation of N-(3,5-dinitrobenzoyl)-DL-amino acids over (R)-N-(11-triethoxysilylundecanoyl)cyclohexyl-(6,7-dimethyl-1-naphthyl)methylamine; L-enantiomers are found to travel more slowly than their isomers in this h.p.l.c. system. The analogous process employing silicates carrying various L-valylamides has been developed further.[397] The remaining major h.p.l.c. resolution technique is represented by further studies of the use of Marfey's reagent,[398] prepared by reacting 1,3-difluoro-4,6-dinitrobenzene with one equivalent of L-alanine amide. DL-Amino acids treated with the reagent yield diastereoisomeric N-aryl-DL-amino acids that are found to be better for the purpose of resolving (RS)-β-leucine than long-standing alternatives such as diastereoisomer formation of the DL-amino acid benzyl ester with (-)-10-camphorsulphonic acid.[399]

As in the last few examples, h.p.l.c. methods have been applied most often to derivatized amino acids rather than to the amino acids themselves. This is related to raising a colour response of one sort or another so that high sensitivity can be achieved by the h.p.l.c. detector, or it may be because the amino acid derivatives have arrived in derivatized form in any case, as they would from a sequencing procedure, for example. Nevertheless, post-column derivatization continues to be favoured by some workers, and is mandatory for ion-exchange methods and the increasingly interesting ion-pair methods (for determination of hydroxyproline in tissue fluids, using ninhydrin as post-column reagent and monitoring at 440 nm,[400] for fully automatic routine amino acid analysis using dodecyl sulphate for pairing,[401] and for a broad range of amino acid analyses[402]).

Pre-column derivatization has been reported on, concerning dansyl-[403,404] and dabsyl-[404,405] amino acids, dinitrophenylamino acids,[406] phenylthiohydantoins,[407,408] and other derivatives arising from sequencing studies.[408] The o-phthaldialdehyde - thiol condensation products have become particularly widely used;[409] the method has been used to determine relative amounts of glutamic acid, pyroglutamic acid, and glutamine in samples [410] and in an interesting application in which N-acetylglutamic acid is separated from other materials by ion exchange and subjected to aminoacylase-catalyzed hydrolysis, then derivatized.[411] The h.p.l.c. of N-acyl-, N-alkoxycarbonyl amino and imino acids has been surveyed.[412] Other o-phthaldialdehyde - thiol derivatization studies include its coupling with electrochemical detection[413] rather than the usual fluorescence measurement, its use in an assay for N$^{\varepsilon}$-methyl

lysine,[414] and related studies,also including the use of 3-mercaptopropionic acid as the thiol component of the reagent,[415] and for the estimation of cysteinesulphinate, hypotaurine, and taurine.[416] Attempts are being made to improve this method and extend its scope, notably its sensitivity (use at 1.5 picomole level has been described[417]) and reproducibility. In the latter context, o-acetylbenzaldehyde has been found to give more stable isoindoles in the procedure as it is usually applied, in comparison with o-phthaldialdehyde. Further results will be welcome if they indicate that improved methodology can be established. A two-step procedure (iodoacetic acid followed by the o-phthaldialdehyde reaction) for the derivatization has been proposed,[418] though the benefits do not seem obvious. Bearing in mind that the o-phthaldialdehyde procedure is specific for amino acids, further studies have been described of a two-tier strategy that allows the unreacted imino acids to be determined after all the amino acids have been derivatized. This can be accomplished by the use of 4-chloro-7-nitrobenzo-2-oxa-1,3-diazole in the case of proline and hydroxyproline, with 3,4-dehydroproline used as internal standard,[419] or by the use of 9-fluorenylmethoxycarbonyl chloride.[420]

Pre-column derivatization by phenyl isothiocyanate has been widely adopted and well supported. The essence of the method is the rapid mild conversion of amino acids into phenylthiocarbamoylamino acids and efficient h.p.l.c. analysis.[421]

7.5 Fluorescence Methods.- Introduction of specific fluorescence methods that have been tested through h.p.l.c. regimes has been announced for 5-pyrrolidone-2-carboxylic acid (derivatized with 4-bromomethyl-7-methoxycoumarin) [422] and for amino acids (derivatization with anthryldiazomethane).[423]

7.6 Other Methods.- Chromatographic methods that are not met in the amino acid context very often have been used for estimation of phenylthiohydantoins (electrokinetic chromatography, i.e. micellar solubilization and electrokinetic migration in a capillary)[424] and for determining binding constants for interaction of L-tryptophan with serum albumin (size exclusion chromatography).[425]

7.7 Determination of Specific Amino Acids.- Comprehensive coverage by the third edition of 'Methods of Enzymatic Analysis' continues to become available, the most recent Volumes including L-serine[426] and S-adenosylmethionine[427] (among many other amino acids). Other enzymatic assays appearing in the primary literature include homocysteine[428] and carnitine (in serum[429] as such, and in esterified form[430]). A rapid enzymatic method for the estimation of D-amino acids[431] employs D-amino acid oxidase and spectroscopic assay of the resulting keto-acids as their hydrazones.

Estimation of the relative amounts of lysine and lysinamide has been based on selective reaction of the former with furfural.[432] A radiometric assay has been reported for guanidinoxy-[^{14}C]-L-canavanine that allows the incorporation of [^{14}C]-canavanine into protein to be measured.[433]

Colorimetric methods are increasingly out of place for routine amino acid assay, beside the sensitive instrumental techniques described in the preceding pages. The red product formed between tyrosine and 4-aminophenazone and $NaIO_4$ in NH_4OH can be estimated spectrophotometrically (λ_{max} 470 nm), allowing the presence of this amino acid in hydrolysates to be determined within $\pm$1-5%.[434] A similar procedure for tryptophan (p-dimethylaminobenzaldehyde, λ_{max} 590 nm)[435] has featured in earlier volumes of this Specialist Periodical Report. Voltammetric[436] and enzyme electrode methods[437] have been described for estimation of tryptophan.

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